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THE PHYSIOLOGY OF THE GALL BLADDER

A. C. IVY

Department of Physiology and Pharmacology, Northwestern University Medical School

During the past decade, various problems pertaining to the activities of the gall bladder and the escape of bile into the intestine have been attacked with enthusiasm and diligence by a large number of investigators throughout the world. New methods have been devised and crucial experiments performed, the results of which have contributed much toward separating fact from fancy in this difficult field. During this period we have witnessed an epoch in the advance of our knowledge of the physiology of the gall bladder.

No really new activities and functions have been discovered that had not been suggested before. But certain hereftofore suspected and implied activities have been definitely established by recent critical work.

It is certain that the gall bladder manifests the principal general activities of the intestine, namely, absorption, secretion and motor activity. The absorption is selective, consisting chiefly in the abstraction of water and certain inorganic salts. The secretion is mucoid in character; the amount of enzymes present, unlike in intestinal secretions, is insignificant. The motor activity is of the general functional types found in the intestine, namely, propulsive and non-propulsive. The biliary ducts have secretory, but little absorptive activity and may or may not manifest motility, depending on the species

A more detailed account of the physiology of the gall bladder will be given under the heading of its three known activities and their functional significance.

ABSORPTION. *Anatomy.* Since the gall bladder absorbs and secretes, one might expect to find on histologic examination two types of cells, although a bipolar cell might serve both functions (207). In fact, two morphologically distinct types of cells in the gall bladder epithelium

(cat, sheep, armadillo and human) have been described (292) (550) (316), one, a typical columnar cell, the other a slender elongated cell. Since intermediate cell types were found, it was suggested (292) (550) that the two types of cells represent functional stages of the same cell, the slender elongated cell being a columnar cell which has discharged its secretion. Winkenwerder (550), on studying the absorption of ferrocyanidecitrate solution, found that the cells manifest an unequal permeability, the slender cells being markedly permeable, and the columnar cells only slightly so. He rightly points out that studies on absorption by the gall bladder should be accompanied by microscopic investigation, particularly in view of his finding that relatively non-toxic solutions may injure the epithelium and that mere trauma to the gall bladder incident to injection may be sufficient to produce abnormal phenomena of absorption. Aschoff (22) states that even normal saline solution may irritate the mucosa of the gall bladder.

Because of the relatively rich lymphatic supply of the gall bladder and because of the possible rôle the lymphatics may play in the absorptive process, particularly in regard to lipoids, a brief statement in regard to the distribution of the lymphatics to the mucosa of the gall bladder is indicated.

Using a more adequate method than was available to the earlier investigators (463) (496), Winkenwerder (550) has found in the subepithelial layer of the gall bladder of the cat a delicate plexus of anastomosing lymphatic channels devoid of valves. This plexus does not give off branches which extend into the stroma of the mucosal folds. Only blood capillary tufts are found in the mucosal folds. Hence, substances, in order to gain entrance to the lymphatics, must seep through considerable stroma or be carried by wandering cells. This indicates that substances absorbed by the gall bladder epithelium pass primarily into the blood vascular, and not into the lymphatic system. In this connection, it should be pointed out that lymph follicles are sometimes found in the folds of mucosa. The size and number of follicles are subject to species and individual variation (496). They are particularly evident in the dog and undergo marked hyperplasia under certain conditions (524) (525).

Concentrating activity of the gall bladder. In 1728, Heister (238) pointed out that gall bladder bile is more viscid than hepatic bile (199) (306) (376). Bidder and Schmidt (47) (48) were apparently the first to determine the dry residue of hepatic and gall bladder bile obtained experimentally in a number of animals. They found the bladder bile to

be four to five times more concentrated. Since then, many (49) (79) (189) (230) (231) (254) (499) comparative chemical analyses of gall bladder and hepatic bile have been made on animals and man, all of which show that the normal gall bladder concentrates from six to ten times the hepatic bile that enters it.

This problem was not attacked by a direct experimental method until 1921 when Rous and McMaster (456) devised a method in which a bile of known composition is supplied through the bile passages to an intact gall bladder by the animal's own liver. This was accomplished by taking advantage of the anatomic arrangement of the bile ducts in the dog. A cannula, to which was attached a rubber bag, was inserted into the common duct for the collection of a portion of the hepatic bile. A partitioning ligature was then placed so that a portion of the hepatic bile could also flow into the intact gall bladder. After certain periods of time, the pigment content of the gall bladder bile was compared with the hepatic bile in the bag, since it had been shown that bile coming from different portions of the liver at the same time had approximately the same pigment concentration. Thus were obtained definite quantitative data on the concentrating ability of the gall bladder. This method was improved later by McMaster and Elman (380). It was found that the gall bladder of the dog may concentrate the hepatic bile from six to ten and eight-tenths times within twenty-four hours and that the mere passage of bile through the gall bladder may lead to a two and three-tenths to four and eight-tenths fold concentration. This was confirmed by Delrez and Cornet (146). Ravdin (441) reports a concentration of from five to seventeen times. Brugsch and Horsters (86) report that concentration ceases when the total solids reach 20 per cent; others report an upper limit of 25 per cent (19). No greater concentration of the bile was found at forty-eight hours than at sixteen or twenty-four hours, indicating a limit of the concentrating ability. Since absorption occurs rapidly, any single portion of bile should reach the limit in a few hours.

The exact quantitative composition of the fluid absorbed by the gall bladder during concentration of the bile cannot be stated. Brand (79) found that the depression of the freezing point of gall bladder and hepatic bile is the same as that of blood serum. On the basis of comparative analyses, he calculates that concentration is affected by the absorption of a normal saline solution. The inorganic salts are decreased, and the substances of large molecular size are increased correspondingly. This has been confirmed by others (230) (441) (494) (510) (529). The recent data of Ravdin, Johnston, et al. (441) indicate that the "freezing

point of gall bladder bile may be accounted for approximately by the osmolar concentration of base, chloride and bicarbonate present."

Considerable work, mostly on the dog, has been done within recent years on the absorption by the gall bladder of the various natural constituents of bile and of foreign substances.

Absorption of water. According to Brugsch and Horsters (86), the maximum rate at which water is absorbed by the gall bladder is 6 cc. per hour. Ravdin et al. (441) who have used a method relatively free from objection report a rate of 6.75 cc. per hour. In another paper, Johnston, Ravdin et al. (280) observed an average hourly rate of 3 cc. They found a diurnal variation, the average rate during the night being 3.7 cc. and during the day, 1.7 cc. per hour. The diurnal variation is related to the activity of the animal. Meals had no effect on the rate of absorption. These statements also apply to sodium chloride solutions.

Change in reaction of bile during concentration. Many observations (79) (117) (127) (157) (190) (251) (374) (375) (409) (414) (420) (437) (426) (441) (506) (529) have been made on the reaction of hepatic and gall bladder bile in different animals. The results of comparative tests indicate that as the gall bladder concentrates the bile, there is an increase in acidity. By using direct methods, Drury, Rous and McMaster (157) and Ravdin, Johnston et al. (441) have unequivocally demonstrated this indication to be a fact. The former observers obtained an average pH of 8.20 for liver bile (dog). The same bile after being concentrated by the gall bladder had a pH of from 5.18 to 6.00. The latter observers introduced dog's liver bile having a pH of from 7.1 to 8.6 into the gall bladder. After five hours in the gall bladder, the pH of the bile was increased to from 6.3 to 7.5 respectively.

Gamble and McIver (199) have studied the total base and chloride of liver and gall bladder bile in the cat. They found the fixed base of gall bladder bile to be twice that of hepatic bile and the chlorides to be almost entirely absent. A more complete study of the anion-cation changes has been made by Ravdin, Johnston et al. (441) in which the anion-cation content of bile before and after being subjected to the action of the gall bladder was determined. The results showed, first, that when the empty gall bladder concentrates hepatic bile, there occurs an absorption of both base and determined anion (chloride and bicarbonate), and an increase in the concentration of the undetermined anion (probably fatty acids and bile acids), and, second, that a "selective" rate of absorption of the various ions occurs (see Tschopp, 510).

Absorption of chlorides, of bicarbonate and of sodium. The absorption

of chlorides by the gall bladder is definitely shown by the comparative chemical analyses of numerous investigators. Recently, Ravdin, Johnston et al. (440) have studied the absorption of chlorides by introducing various concentrations of NaCl in aqueous solutions into the empty gall bladder. They also used a sodium chloride-sodium bicarbonate (total anion 136 m. Eq. per liter) solution. Regardless of the tonicity of the solution, chloride was absorbed. Chloride was absorbed even when the gall bladder in response to a hypertonic solution was releasing water into its lumen to render its contents isosmotic with the the blood serum. It should be noted that repeated introduction of hypertonic saline damaged the gall bladder mucosa as indicated by the slow absorption of water, excessive secretion of fluid, and histologic changes. In the cases of isotonic solutions, chloride and water left at about the same rate. In hypotonic solutions the water left faster than the chloride so that the fluid tended to become isotonic. As the chloride left, it was replaced chiefly by bicarbonate, only traces of carbonate and phosphate being found. When the sodium chloride-sodium bicarbonate solution was introduced, the water, sodium, chloride and bicarbonate were absorbed at approximately the same rate. It is evident that the sodium (254) (510), chloride and bicarbonate ions are absorbed by the normal gall bladder. Bicarbonate may pass through the normal mucosa in both directions. The tendency is for chloride to pass into the blood only, except when the damaged mucosa begins to pour in fluid, then chloride passes from the blood.

Absorption of calcium. The influence of the gall bladder on the calcium content of bile has been subjected to considerable debate. The older data (254) are of such a nature that they cannot be used, although they indicate that calcium may be absorbed. The more recent observations of Drury (158), Mirvish, Sacks and Schrire (393) and Andrews and Hrdina (18) show that calcium may be absorbed, although they are open to some objection on the basis of the experimental method employed. Johnston et al. (280) have reported interesting observations on the absorption of calcium lactate (3 to 100 m. Eq. per liter) introduced into the bile free gall bladder by a method which does not interfere with the blood and lymph supply or traumatize the gall bladder. They found that calcium was absorbed. During the day, the calcium concentration was increased, showing that water was absorbed faster than calcium; but during the night, the opposite occurred. When the gall bladder was damaged, calcium was absorbed at a much faster rate than water so that the concentration decreased from the start. When the

gall bladder was damaged to the extent that fluid flowed freely into the gall bladder, calcium was excreted. When the gall bladder was so damaged that water was not absorbed, a histologic examination showed calcium deposits in the submucosa (425). The lactate ion was absorbed more slowly than the calcium ion and did not show a diurnal variation in concentration. There was a tendency towards rendering the total osmolar concentration of the contents equal to that of serum either by concentration or by the excretion of base. The base excreted was chiefly sodium. The anion balance was maintained primarily by the passage of chloride and carbonate, chiefly the former, from the circulating fluids. The sum of the anions approximated the total base. Experiments in which the method of Ravdin et al. (280) is used and in which hepatic bile is introduced, should be performed so as to avoid damage to the gall bladder by foreign solutions. However, it is quite evident from the data at hand on the cat and dog that calcium is absorbed by the normal gall bladder, that *calcification of the wall of the gall bladder has been produced experimentally*, and that a damaged gall bladder mucosa may excrete fluid containing calcium.

Absorption of pigment. That bile pigment may pass from the bladder contents into the circulation when the cystic duct is obstructed for a period of weeks is well known. But such evidence, particularly in view of the anatomic changes that so frequently result (17) (258, and others) does not show that pigment is absorbed in appreciable quantities under normal conditions. That some pigment under normal conditions may pass through the mucosa is apparently indicated by it being observed histologically in transit (23). The results of Rous and McMaster (456) indicate that the absorption of pigment under normal conditions is negligible, although the pigment found in the gall bladder frequently fell short of the expected quantity. These observers and also Riegel, Johnston and Ravdin (450) have cannulated the lymph duct draining gall bladder and have failed to obtain a test for pigment. Sweet (497), however, obtained positive tests for bile pigment in gall bladder lymph. Ravdin and Morrison (442) obtained negative tests for bile pigment in the venous blood of the gall bladder. The most decisive data reported on this question are those of Riegel, Johnston and Ravdin (450). Known hepatic bile was introduced into the gall bladder by their method. The bile was permitted to concentrate for periods ranging from two to twenty-four hours. The median loss of pigment was 4.8 per cent (maximum loss 3 mgm. in 24 hours), which they accounted for by experimental error. Even though this loss were real, it is so small as to be practically negligible.

Absorption of bile salts. Because of the lack of a satisfactory method for the quantitative estimation of bile salts, all observations reported on this subject can be considered as only roughly comparative. On comparing the results on the bile salt concentration of fistula bile with that of gall bladder bile reported in the literature, a conclusion is impossible. Some of the results indicate absorption, others, the contrary. Fox (189) summarizing the literature up to 1927, concludes that, considering the inaccuracies, the difference in bile salt concentration of fistula and bladder bile is mainly, if not entirely, one of concentration. The more recent indirect results of Rosenthal and Zinner (453) on man indicate that bile pigment is concentrated more than bile acids by the gall bladder. The comparative results of Aldrich and Bledsoe (11) indicate a greater concentration of bile salt in the dog than in the human gall bladder. That cholates may pass into the gall bladder lymph of the dog was demonstrated by Rous and McMaster (456), since they occasionally obtained a positive Hay's test. Rosenthal and Licht (452) aseptically ligated the cystic duct of the dog, withdrew the bile through a needle puncture, washed out the gall bladder, introduced a portion of the bile withdrawn, and closed the puncture with a stitch. The gall bladder contents were then examined for bile salts from two to ten days later. They observed an absorption of bile salts amounting to from 6 to 8.7 per cent per day. Their method is open to the criticism cited before. In cats with the cystic duct ligated, Hunt, Davis and Boyden (258) found bile salts present in the gall bladder after two weeks. Riegel, Ravdin and Johnston (449), who used a better experimental method and one which permitted them to ascertain if the gall bladder were concentrating and if the solution caused damage, introduced a bile salt solution into the gall bladder. Blood was obtained constantly from the fluid after from five to twenty-four hours, a fact that has been confirmed in the author's laboratory after the introduction of bile salt solutions. So, the duration of their experiments was limited to from two to six and one-half hours. Water was absorbed, and the bile salt was concentrated and reduced in total amount. The average absorption of bile salt was 20 per cent. Since bile salt solutions are so toxic for the gall bladder mucosa, this figure cannot be considered as normal, and cannot be considered as indicative of normal as the figures supplied by Rosenthal and Licht (452). The use of Ravdin's method and the introduction of hepatic bile into the gall bladder should yield more trustworthy data. Considered as a whole, the author interprets the available data as indicating that small quantities of bile salts may be absorbed by the normal gall bladder.

The literature is agreed on the point that inflammation or irritation of the gall bladder increases the rate of absorption of bile salts (16) (20) (152) (411) (443) (449).

Absorption of cholesterol. Much discussion has been centered on the question of whether cholesterol is absorbed, secreted, or only concentrated by the gall bladder; and considerable investigation has been directed toward answering it. The literature up to 1929 has been reviewed by Lichtwitz (334) and space does not permit a thorough discussion of biliary cholesterol in this review.

The importance of the problem is obvious to every student of biology. The problem is complicated by the species variations in the cholesterol and lipid content of bile. (For a recent summary of the subject of cholesterosis of the gall bladder, the reader is referred to an article by Illingworth (259).)

In 1857 Virchow (515) observed histologically in the gall bladder mucosa a lipoid which he considered to be cholesterol. Since then, many microscopic studies have been made of cholesterol in the mucosa. The difficulty with this method is that one cannot be certain in which direction the cholesterol (cholesterol esters or loose combinations of cholesterol and fatty acids) is passing. The question of interpreting the observed facts soon resulted in two schools. One, the Naunyn (406) school, which maintained that cholesterol was excreted (desquamated as a result of inflammation) by the mucosa; the other, the Aschoff (23) school, which maintained that cholesterol and fats are absorbed. Using histologic methods, it has been demonstrated that an increase in neutral fats or fatty acids may be detected in the gall bladder mucosa after introduction into the gall bladder; but an increase in cholesterol has not been demonstrated, although much work has been done (24) (259) (275) (388).

The comparative analyses of gall bladder and hepatic bile obtained from different sources are of questionable value in answering the question because of the wide variations (182) (189) and the conditions under which the bile samples have been obtained. The data prior to 1909 should hardly be considered because of the inadequacy of the cholesterol methods available. Fox (189) has summarized the cholesterol analyses of human fistula and bladder bile reported between the years 1909 and 1929. From comparing the increase in cholesterol in bladder bile with the increase in total solids, he concluded that the difference in content of cholesterol in the two biles is due only to the degree of concentration, indicating neither absorption nor excretion. In this connection, however, it must be kept in mind that the gall bladder through its mucoid secretion adds solids.

Direct experimental evidence on this question is limited and some of it is open to objections. Torinoumi (507) ligated the cystic duct, withdrew and measured the contents of the gall bladder, kept a portion for analysis, and returned the remainder to the gall bladder. He sacrificed the dogs from one to four weeks later. In three he found a decrease in cholesterol. In two in which an increase was found, inflammation was present. In similar experiments, Elman and Taussig (173) (174) report evidence of absorption in only one of nine dogs. In the remainder, a slight to definite increase was obtained, inflammation being found in only two instances. Illingworth (259) in two cats ligated the cystic duct and introduced cat's bile to which he added a large amount of cholesterol. After five days he found an absorption of more than fifty per cent, concluding therefrom that cholesterol when present in excess in certain physical mixtures can be absorbed in a form not demonstrable histologically. The absorption amounted to only 4 mgm. per day approximately. The objection to this work has been made that the cholesterol was probably removed by phagocytosis (173), and that a statement was not made concerning the possibility that some of the cholesterol settled out and was not removed from the mucosal surface (450). Delrez and Cornet (146) placed a known amount of cholesterol in mineral oil and introduced it into the bile free gall bladder of dogs with the cystic duct ligated. After two days, they recovered an increased amount of cholesterol. Elman and Taussig (173) repeated these experiments with the same results. Westphal, Gleichman and Soika (529) studied the effect of various procedures on absorption in anesthetized dogs with the cystic duct tied. They obtained samples of bile by puncturing the gall bladder through the liver. Under vagus stimulation for periods of from 120 to 150 minutes, they observed concentration of the bile pigment without a similar concentration of cholesterol which, as they concluded, indicated a slight but definite absorption of cholesterol. Wilkie and Doubilet (541) tied the cystic duct of dogs, removed the contents by needle, avoiding saline washings because of possible injury, and introduced dog's hepatic bile of known cholesterol content. In some experiments they added cholesterol to the bile, as in Illingworth's experiments. Their data indicated that cholesterol may pass both ways through the gall bladder mucosa, depending on the blood-bile cholesterol ratio. If the bladder bile cholesterol was high, cholesterol was absorbed. If the bladder bile cholesterol was low, then cholesterol was excreted. Elman and Taussig (173) removed the gall bladder at operation (man and dog) and then obtained hepatic bile by a cannula and compared the pigment and choles-

terol concentrations in the two biles. They found the cholesterol content of the bladder bile greater than could be accounted for by pigment concentration, and concluded that the gall bladder excretes cholesterol. They reported some data favoring an increased excretion of cholesterol in cholecystitis. Andrews, Schoenheimer and Hrdina (19) using the same method as Elman and Taussig but comparing the bile salt cholesterol ratio instead of the pigment cholesterol ratio, came to the conclusion that a differential absorption of bile salts and cholesterol does not occur. They believe that their data indicate that neither bile salts nor cholesterol are absorbed by the normal gall bladder. In regard to Elman and Taussig's results, and those of others who have reported excretion of cholesterol, Andrews, Dostal and Hrdina (15) have pointed out that the escape of blood (resulting from a needle puncture) into the bile might account for the observed increase in cholesterol. Another objection is that the hepatic bile collected, in regard to cholesterol content, may not be an accurate index of the bile previously secreted, i.e., before the anesthetic, and concentrated in the gall bladder. This objection is tenable because McMaster (378) has reported that pigment output is relatively constant when compared to the rather abrupt and considerable fluctuations of cholesterol output. Again one must be cautious in making such comparisons, because the pigment output is subject to variations (note some of the data of Drury (157) (158)). Elman and Graham (171) have performed a series of experiments that appear more significant in regard to the excretion of cholesterol by the gall bladder. In dogs they placed a partitioning ligature so that a portion of the hepatic bile was diverted into the gall bladder, producing an obstruction to the portion of the liver drained. The remainder of the hepatic bile was collected in a bag. The duration of the experiments was forty-eight hours. The pigment and cholesterol content of the two biles per gram weight of liver drained was compared. In five experiments the bile coming into contact with the gall bladder contained more cholesterol than the other. In the experiment that gave the greatest increase in cholesterol (153 per cent), the gall bladder was inflamed. An increase of 122 per cent was observed in another experiment in which the gall bladder was grossly normal but sections were not made. The data are too few, however, to convince one, particularly in view of the possibilities that the bile secreted per gram of liver may not be the same under their experimental conditions, that at the operation the gall bladder may not have been completely emptied of its bile by the needle used, and that the puncture of the gall bladder through the liver may have introduced

blood containing cholesterol. Riegel, Ravdin and Johnston (449) introduced an aqueous suspension of cholesterol into the bile free gall bladder of dogs by the method cited before. Water was absorbed; the cholesterol was concentrated and settled out of the suspension. Their results were variable (+3 to −32 per cent) but were explained as due to an experimental error, the error being their inability to wash out all the cholesterol at the end of the experiment (7 to 105 hours). Later, these observers (450) introduced sterile hepatic bile from the same dog into the gall bladder where it was permitted to remain for from five to thirty hours. The mean loss of cholesterol was 10.5 per cent. They interpreted their results as indicating that neither secretion nor absorption of cholesterol occurs in the normal gall bladder, believing apparently that the loss observed could be accounted for by experimental error, since the largest per cent loss actually amounted to only 1.8 mgm. of cholesterol. They also introduced bile containing 12 mgm. of cholesterol into an infected gall bladder and after twenty-four hours removed 23.5 mgm.

Riegel, Ravdin and Johnston (449) have reported data on the changes occurring in the gall bladder when an aqueous bile salt suspension of cholesterol is introduced into the dog's gall bladder (concentration of sodium taurocholate 3–8.7 mgm. and of cholesterol 0.3–1.7 mgm. per cc). This mixture had the advantages that cholesterol was not so likely to settle out from suspension in the gall bladder and that hemorrhage or acute irritation did not appear until twenty-one to one hundred ninety hours later. In the experiments, the bile salt cholesterol mixture was permitted to remain in the gall badder an average of twenty-nine hours. Water and bile salt were absorbed in all experiments, but the absorption of bile salt was considerably less than that which occurred when bile salt alone was used. Although some cholesterol was lost, the data were interpreted as indicating that if some cholesterol were actually physiologically absorbed, the amount was too small to be of much significance. When the gall bladder mucosa was damaged, cholesterol was excreted. (For a consideration of the bile acid cholesterol ratio in the bile of damaged gall bladders in dog and man as related to gall stone formation theories, the reader is referred to references (19) and (411).)

It is obviously difficult to evaluate the data on cholesterol absorption. It appears, however, that the data most free from objection indicate that the normal gall bladder mucosa absorbs relatively small amounts of cholesterol daily, if any, and that the inflamed mucosa, because of its cholesterol content, may add cholesterol to the gall-bladder contents by desquamation. The pathologic significance of this latter process is certainly open for further investigation.

Absorption of neutral fats. The view that neutral fat may be absorbed by the gall bladder was first advanced by Virchow (515) on the basis of finding fat droplets histologically in the mucosa of the human gall bladder. This has been confirmed by others (22) (23) (24) (59) (225) (427). Aschoff (23) (24) introduced olive oil, butter, and sterile milk into the gall bladder of dogs in which the cystic duct was ligated. After a few days, lipoid deposits were found in the epithelial cells. Controls with a ligated cystic duct did not show such deposits. A number of observers (147) (178) (316) (388) (458), employing practically the same method and introducing various forms of neutral fat, have confirmed the original observation of Aschoff. Whitaker (538) filled the gall bladder of the cat and dog with iodized sesame oil (lipiodol) and caused it to remain in place by fasting the animal, rather than by tying the cystic duct. After several days, much fat was found in the epithelium. After several weeks, it had disappeared from the epithelium. In regard to this observation, the criticism might be raised that iodized oil is mildly irritating at times to the gall-bladder mucosa, but Whitaker states that although there was some increase in lymphocytes in the stroma of the epithelial folds, the tissues on the whole were remarkably free from inflammatory reaction. The evidence shows that fat may pass through the epithelial cells under the experimental conditions employed to date, but there is no evidence showing that bile fat passes through the normal gall bladder mucosa during the concentration of bile.

Everyone, with the exception of Mentzer (388), has reported that the fat which passed the epithelium in the above experiments entered the lymphatics. The fat has been described as being in the lymphatic capillaries of the mucosal folds and in the deeper lymph trunks. Winkenwerder (550), who was unable by improved methods to find lymphatics in the folds of mucosa, believes that venous capillaries were mistaken for lymph capillaries. Mentzer (388) was unable to find fat in the lymphatics or in the lymph nodes into which the bladder lymph drains and concluded that the fat passed into the veins.

Tschopp (510) has reported that the cholesterol-lecithin ratio remains constant during concentration of the bile.

Effect of drugs and stimulation of nerves on absorption. Westphal, Gleichman and Soika (529) tied the cystic duct, obtained a sample of the bladder bile for analysis and then observed the effect of certain drugs and of stimulation of certain nerves on the concentration of bile. They concluded that atropine does not prevent normal absorption or

normal concentration. Stimulation of the vagus peripherally increased the rate of concentration. Stimulation of the splanchnic decreased the rate of concentration. They believe the changes in rate of absorption to be related to changes in the motor activity. Vascular changes must obviously be considered in the interpretation of these results. They suggest that gall bladder resorption in man may fluctuate under nervous influences.

"Absorption" of bacteria. Lange and Roos (321) injected bacilli into the gall bladder of the rabbit and report the recovery of the same bacilli from the ear vein in two minutes! Nedzel and Arnold (408), after withdrawing the bile, introduced bacillus prodigiosus (a small, motile coccoid bacillus) in saline solution into the gall bladder of anesthetized dogs. The solution was introduced via a catheter passed up the common and cystic ducts into the gall bladder. The catheter was tied in place by passing a ligature about the cystic duct close to the common duct without interfering with the blood supply. After thirty minutes, they recovered the bacilli from various parts of the liver. Sterile gauze placed about the incision in the common duct, was negative to culture in six of ten experiments.

Absorption of inert particles. Mentzer (388) has reported that within a few minutes (!) after the introduction of finely powdered charcoal and India ink into the lumen of the gall bladder, particles of these substances could be found in the lymphatic structures. Cooke (120) could not confirm this report.

Absorpion of dyes and crystalloids foreign to bile. A number of investigators in studying the problem of absorption have used various dyes and crystalloids foreign to bile.

Boyd (59) and Winkenwerder (550) have shown that the Prussian blue reagents (ferric ammonium citrate and potassium ferrocyanide) pass through the epithelium when introduced into the lumen of the gall bladder. Boyd reported that to obtain the best results, the gall bladder had to be washed out, and Winkenwerder found that these reagents would injure the epithelium if permitted to remain in contact from thirty to forty minutes.

Several investigators (49) (243) (497) have observed absorption of potassium sulphocyanide. Reactions for its presence in the lymph draining from the gall bladder have been obtained (243) (497).

Potassium or sodium iodide, which are absorbed by every mucous membrane, are absorbed quite rapidly from the gall bladder (123) (275) (281) (499).

The following dyes have been reported to be absorbed by testing the urine, hepatic bile, or lymph drainage of the gall bladder: Indigo-carmine (275) (388) (533); phenolsulphonphthalein (275); methylene blue (275) (446). Rewbridge, Hanke and Halpert (446) found methylene blue to be absorbed rather slowly from the dog's gall bladder, too slowly to account for its disappearance from the gall bladder in twenty-four hours. Nakashima (403) used a number of dyes of varying diffusibility and found that diffusibility and not chemical structure determined the rate of absorption. Igwanaga (275) reported that inflammation increased the rate of appearance in the urine of the dyes he studied (phenolsulphonphthalein, methylene blue, potassium iodide, and Indigo-carmine). He concluded from his studies that the gall bladder acted like a semi-permeable membrane.

Absorption of tetraiodophenolphthalein. Whether tetraiodophenolphthalein is absorbed from the gall bladder deserves special consideration, since some (54) (499) have maintained that the disappearance of the gall bladder shadow after a fat meal means absorption of the dye.

A number of clinical reports on cholecystography include the statement that as the gall bladder empties, the cholecystogram becomes more dense (386) (485) due to an increase in absorption of water. This is not always true, however, as a review of the extensive cholecystographic literature will demonstrate (106) (211). If the dye is absorbed by the human gall bladder, it must be relatively slowly, since in fasting or on the ingestion of carbohydrate meals, the shadow may persist for twenty-four hours or longer (106) (132) (211) (485). Of course, a fat meal by increasing the tonus rhythm of the gall bladder or in some unknown way may increase the rate of absorption. But Johnston (281) claims that in the dog a fat meal does not increase the absorption of the dye. In this connection, Menees and Robinson (387) report a case in which after the gall bladder was visualized with the dye, the cystic duct became obstructed with a stone and the shadow persisted for twenty-one days.

Copher, Kodama and Graham (121) (126) found that tetrabromphthalein is concentrated from two to four times in the gall bladder at maximum density. Lomon (loc. cit. (112)) reported a similar concentration of tetraiodophenolphthalein. Data which we (518) have obtained from the dog show that iodine may be concentrated from three to six times or to about the same extent as the concentration of pigment observed at the same time. Sweet (499) performed an interesting experiment on two dogs, in which a known quantity of dye was introduced

into the bile free gall bladder, and an hepatic duct was cannulated so that its bile drained into a bag, the remainder of the hepatic bile being shunted into the gall bladder. The rationale of the experiment was that if the gall bladder absorbed the dye, after a period of time it would all be found in the bag. After twenty-four or twenty-six hours, he found approximately 66 per cent of the dye, which had been introduced into the gall bladder, in the rubber bag. This indicated an appreciable absorption of the dye. Johnston (281) repeated and extended Sweet's experiments. He concluded that sodium tetraiodophenolphthalein is absorbed more slowly than sodium iodide, that a fat meal does not increase the rate of absorption and that the rate of absorption of tetraiodophenolphthalein is so slow that it does not seem possible for absorption to account for the rapid disappearance of the gall bladder shadow seen in cholecystography. These results of his experiments would have been more significant had the dye been dissolved in bile, and had it been shown that the dye in the solutions used did not injure the gall bladder mucosa. Delario (145), on studying the elimination of the dye by the liver, has reported that with the gall bladder present, the dye reaches its maximum concentration in the bile in seven hours, but requires only five hours in the absence of the gall bladder. He interpreted his observations (not stating the number of animals used), as indicating that the gall bladder by absorbing the dye, prolongs the elimination of the dye by the liver.

It appears from the literature that the gall bladder absorbs tetraiodophenolphthalein at a slow rate. However, further data, obtained by more adequate experimental methods, are necessary before the rate of absorption which occurs under the practical conditions of cholecystography can be established. At least, it has not been demonstrated that the dye is absorbed at a rate sufficient to account for the disappearance of the gall bladder shadow after a fat meal. The evidence to be cited later shows that after a fat meal, the major portion of the dye in the gall bladder is evacuated through the cystic duct.

Absorption in cholecystitis. Afanassiew (4) found that jaundice developed more rapidly when the biliary tract was filled with wax than when the common duct was tied. Mann and Bollman (360) observed by following the bilirubin of the blood and urine in dogs with common duct obstruction that dogs with a gall bladder develop jaundice more slowly than cholecystectomized dogs. Using this fact as a test of gall bladder concentration in dogs in which cholecystitis had been produced by the intravenous injection of Dakin's solution (358), it was

found (56) that the rapidity with which jaundice developed showed a direct relation to the degree of acute cholecystitis present. Slight cholecystitis did not have much effect; but when the cholecystitis was marked, jaundice developed as quickly as if the gall bladder had been excised. Darnbacher and Sacks (140), by using cholecystography and determining the specific gravity of bile, found that acute experimental cholecystitis in the dog may or may not prevent concentration depending on the nature, extent and location of the lesions. Direct irritation of the entire mucosa resulted in a loss of concentrating ability. Acute inflammation of the deeper layers of the gall bladder wall caused no immediate decrease in concentration but later the gall bladder may in some instances lose its concentrating ability. These investigators and also Murphy and Bollman (400) found that the concentrating ability may be restored on recovery from acute cholecystitis. Ravdin and others (280) (440) (449) have shown quite definitely that water is not absorbed by the inflamed gall bladder mucosa and that certain changes result in the absorption of calcium (*vide supra*). The absorption of bile salts has been shown to be increased (19) (452) (499). A number of reports or comparative analyses of bile from various types of inflamed and normal human gall bladders have been made. The general trend of these reports shows that bile from acutely inflamed or markedly chronically inflamed gall bladders is less concentrated (107) (182), but the variations are wide. The most convincing observations are those of Caylor and Bollman (107), in which studies of the pathology and chemistry were made in one hundred and five cases. The conclusions were that concentrating ability is lost in acute inflammation, empyema, hydrops (cystic gall bladder), and secondary "contraction" of the gall bladder, and that in the papillomatous gall bladder with hypertrophied rugae, concentration is increased.

Igwanaga (275) has reported that bacterial and mechanical inflammation of the gall bladder mucosa increases the rate of absorption of phenolsulphonphthalein, methylene blue and indigo-carmine. Winkenwerder (550) has demonstrated that a slight injury of the gall bladder causes a massive absorption of the Prussian blue reagents. Since some have made the very unlikely suggestion that the failure to obtain a cholecystogram in some diseased conditions is due to the more rapid absorption of the tetraiodophenolphthalin, studies should be made on the rate of absorption of this latter dye by the inflamed gall bladder.

It should be mentioned that the failure of the gall bladder to visualize in cholecystography is interpreted to be due either to *a*, failure of liver

to excrete the dye adequately; *b*, incompetence of the sphincter of Oddi; *c*, obstruction of the cystic duct, or *d*, such an extensive cholecystitis that the dye is not concentrated in the gall bladder. Much clinical as well as experimental evidence confirms this interpretation.

Absorbing ability of the gall bladder as related to cholesterol metabolism. Sweet (497) has reported interesting data on the blood cholesterol changes induced by a fat meal before and after cholecystectomy. He found that the blood cholesterol after a fat meal rises to twice normal in cholecystectomized dogs. This rise lasts for about forty days, then falls below normal at about seventy-four days. The rise is chiefly due to an increase in cholesterol esters. No change in lecithin was observed. Fowweather and Collinson (188) studied blood cholesterol before and after cholecystectomy in man. The blood cholesterol values several months after the operation were normal, or below, on the average, which because of the time factor is not contrary to the findings of Sweet. Singer (481) failed to find the stercobilin in the feces of patients to be affected by cholecystectomy; this does not mean much, since the gall bladder may have been functionless prior to cholecystectomy. Such observations deserve repetition and extension, because changes in the digestion and absorption of fat and sterols in the intestine may be influenced by cholecystectomy (59) (93) (451) (471) (502), and not necessarily because a metabolically significant amount of cholesterol is absorbed by the gall bladder.

General summary of absorption. It is established that the normal gall bladder concentrates from four to ten times the hepatic bile that enters it. This is accomplished primarily by the absorption of water and inorganic ions according to the law that the gall bladder content tends to remain isosmotic with serum as determined by the freezing point method. In this process, the bile is slightly acidified. The upper limit of concentration of total solids is roughly from 20 to 25 per cent. The other biliary constituents appear to be absorbed only slightly, if at all, by the normal gall bladder. More careful work, and in certain instances better chemical methods than now are available, are requisite to a complete understanding of the physiologic niceties of gall bladder absorption, upon which depends the interpretation of numerous questions of much practical importance.

Absorption from the extra-hepatic bile ducts. Until Rous and McMaster (457) did their work on stasis bile, it was generally assumed that the bile ducts tended to concentrate bile as does the gall bladder. Klee and Klupfel (308), on examining the contents of the dilated common

duct after cholecystectomy, thought that it was more concentrated than normal, although no analyses for bilirubin content were done. Rous and McMaster (457) isolated segments of extra-hepatic ducts and after periods of time up to twelve days examined the contents. During the first few days, the content appeared darker than normal due to the conversion of some of the bilirubin to biliverdin. After several days, the fluid no longer gave tests for bile pigment and cholates. This shows that a slow absorption occurs. However, this is entirely overbalanced by the secretion which may distend the ducts in the dog until they measure as much as 1 cm. in diameter. From these and other basic experiments, they concluded that the gall bladder differs from the ducts in their influence on bile. The ducts instead of concentrating it, dilute it slightly with a thin colorless secretion devoid of cholates, even in the presence of marked jaundice. They, however, made no analyses of the bile in the ducts during the first few hours. Brugsch (86) in some indirect experiments, in which the amount of pigment and refractive index of the bile in the extrahepatic ducts was studied several hours after obstruction, came to the conclusion that the ducts may also concentrate bile. They suggested that bile may be concentrated by the dilated ducts of the cholecystectomized patient. In regard to such cases, it is stated by Judd (288) that concentration is not found. Sufficient concentration does not occur in dilated ducts after cholecystectomy to cause them to become visualized by cholecystography (211) (307) (464). Burden (92), who made an anatomical study of the parietal sacculi of the common duct, thought that it was "no more probable for bile to enter the sacculi and be absorbed than for duodenal contents to enter the glands of Brunner and be absorbed." Direct experiments on the resorptive capacity of the dilated ducts after cholecystectomy have not been made.

Some observers have reported an increase in intracellular lipoid in the epithelial cells after the introduction of neutral fat into the extrahepatic ducts (for literature, see that given for absorption of neutral fat by the gall bladder).

In connection with this subject, McMaster (377) asked the question, "Is it possible that in animals lacking the gall bladder, the concentrating function is lodged somewhere in the duct wall?" He proceeded to answer the question by studies on the mouse and rat, two closely related species, the former having a gall bladder and the latter not. In order to appreciate fully the significance of his results, it should be pointed out that when the common duct is obstructed in animals with a gall bladder,

the duct content becomes concentrated under the influence of the gall bladder; but when the hepatic duct is obstructed, the duct content becomes diluted. In the first place, he found that the hepatic bile of the rat contained eight times more pigment than that of the mouse. In the second place, he found that obstruction of the common duct in the mouse caused the duct bile to become concentrated, whereas in the rat it was not concentrated. In order to ascertain if concentration occurred early in the rat, he killed some of them on successive days, failing to obtain evidence of concentration at any time. It appears to be evident for the rat at least, that to compensate for the morphologic absence of the gall bladder, either the liver secretes a more concentrated bile, or the sacculi of the intra-hepatic ducts of the rat concentrate it (Higgins, (247a)). This statement, of course, applies only to pigment, as analyses for other constituents were not made. Boyden (70) has reported a pigment analysis of the bile in a cat in which the gall bladder was congenitally absent. The pigment was 2.2 times more concentrated than in control cats.

Secretion. The gall bladder forms a mucoid secretion. Whether or not true mucin is secreted has been debated. Some older histologists expressed doubt because of the absence of goblet cells in the mucosa. It is now known that a goblet cell structure is not prerequisite for the secretion of mucus (550).

According to Sobotta and Sommier (483), the appearance of the border of the cells is due to mucin particles which may also be found throughout the greater part of the cell body. A distinctive mucicarmine stain has been obtained by several observers (292) (476), especially in the cells of the crypts and sinuses. This has been more recently confirmed by Policard (428) and Bauer and Hokki (37). The latter investigators found that following the loss of the concentrating function of the gall bladder or after cystic duct ligation, or in a peritoneal graft of gall bladder mucosa, or on irritation of the mucosa, or after cholecysto-gastrostomy, the epithelial cells take on all the characteristics of mucous cells.

Traces of true mucin have been found chemically in the bile of various animals, but the ropy character of the bile is due primarily to a nucleoalbumin similar to mucin. Hammarsten (232) has found true mucin in human bile. But according to Wahlgren (522), the true mucin probably originates in the bile passage, since he was unable to find a true mucin in the gall bladder bile of man. In view of the work of Bauer and Hokki (37), a chemical study of the alcohol insoluble portion

of hydrops bile is indicated. Either true mucin is secreted or the specificity of the muci-carmine stain is to be doubted.

We have no data on the amount of mucoid secretion added to the bile by the gall bladder in the course of a day. The data we possess have come from gall bladder fistula cases in which some disease is or was present or from hydrops bile. Aschoff and Bacmeister (23) have stated that hydrops "is the expression of a change in the bladder wall such that fluid is elaborated by it instead of withdrawn through it." This statement has been affirmed by Rous and McMaster (457). The physiological influences which lead to the production of "white bile" were fully elucidated by Rous and McMaster in 1921, and subsequent work (158) (449) shows that a copious quantity of fluid is poured out by the gall bladder mucosa only when irritated or damaged.

Birch and Spong (50) have reported observations on two patients in which there was an obstruction of the cystic duct with hydrops of the gall bladder and a drainage tube was placed into the gall bladder of which the mucosa appeared normal grossly. They collected about 20 cc. in twenty-four hours, approximately one-third being lost, the quantity being about the same in both cases. In its essential characteristics, the fluid was like hydrops bile, clear to slightly opalescent, almost colorless, viscid, slightly alkaline and free of bile salts. One of the patients was followed for two years and because of the unaltered nature of the fluid as determined by chemical analysis during this period of time, these workers believed that they were observing the normal product of the bladder mucosa. This is the best record that the author has been able to find. Whether it represents a normal secretion is open to question.

In the dog, Rous and McMaster (457) obtained 8.5 cc. of duct secretion in six days from a strip of mucosa measuring 2.0 by 0.7 by 0.8 cm. when the duct was slit longitudinally.

A number of chemical analyses of hydrops, or white bile, have been made, but chiefly in regard to the calcium and cholesterol content. Chlorides and carbonates are constantly found.

Calcium. The finding of "calcium milk bile" in certain cases of cystic duct obstruction in man has been discussed by Volkmann (521) and more recently by Phemister, Rewbridge and Rudisill (425). Dostal, Hrdina and Goff (151) produced this condition in one of a series of dogs, finding the calcium concentration of the fluid to be 580 mgm. per 100 cc. In "white bile" from sterile obstructed segments of the ducts of dogs and from dogs with such marked liver damage that little pigment

was being secreted, Drury (158) obtained calcium concentrations varying from 10 to 12 mgm. per 100 cc. Johnston et al. (280) found in the fluid copiously secreted (2 cc. per hour) by the irritated mucosa, a calcium concentration of from 5 to 10 mgm. per 100 cc. In "white bile" from the human gall bladder, Fowweather and Collinson (188) found from 2.8 to 15 mgm. of calcium per 100 cc., which checks quite well with the calcium content of the "white bile" of dogs. Hence, some additional pathologic factor in addition to simple cystic duct obstruction is necessary to produce "calcium milk bile."

Cholesterol. In the sterile "white bile" from dogs, only traces of cholesterol have been found (457). Non-sterile "white bile" may contain as much as 50 mgm. per 100 cc. (171). In the fluid that is caused to be secreted by slight injury of the mucosa, 5 mgm. per 100 cc. of cholesterol have been found (449). In the hydrops fluid from the gall bladder of patients, Fowweather and Collinson (188) found a cholesterol content ranging from traces up to 130 mgm. per 100 cc. Ellman and Graham (171) found from 19 to 45 mgm. per 100 cc. in two cases.

It is evident that if "white bile" represents a true hypersecretion of the biliary passages, which is not unlikely, only very small amounts of cholesterol and somewhat larger quantities, about that of blood or less, of calcium are excreted by the gall bladder. However, in inflammatory or other diseased conditions, calcium and cholesterol excretion may be greater than that found in clear or slightly opalescent hydrops fluid.

Secretion of dyes and other substances. The evidence at hand does not indicate that the halogenated phthaleins are eliminated by the gall bladder mucosa (112) (121). Mann (359) has performed experiments on the elimination of Rose Bengal which indicate that this dye may be excreted in part by the gall bladder. When Dakin's solution (chlorinated soda) is injected intravenously (358), the toxic effects are chiefly manifest in the gall bladder, various degrees of cholecystitis resulting. Mann (358) showed that this agent acts on the gall bladder by way of the blood stream and not the bile. He suggested that this might indicate a specific elimination. In view of this suggestion, Burgess and Ivy (94) attempted to compare the toxicity of Dakin's solution in normal and cholecystectomized dogs. The results of the experiments (19 dogs) were not conclusive, but gave the impression that cholecystectomized dogs were less tolerant. Copher and Kendall (124) observed cholecystitis to result after the intravenous injection of the fluid from an obstructed loop of intestine.

According to Meyer and Neilsen (391a), the gall bladder mucosa

eliminates to a slight extent bacteria injected intravenously. This results from the general infection of the gall bladder wall and is not specific.

It is probable that under normal conditions, the gall bladder forms only a small quantity of mucoid secretion and that under conditions of acute irritation, it ceases concentrating and pours out a fluid which teleologically may be compared to a type of catharsis.

MOTOR ACTIVITY OF THE GALL BLADDER. *Anatomy.* It is not considered to be in the province of this review to cover the literature pertaining to the gross anatomy (211) (345) and comparative anatomy (354) of the biliary passages and the anomalies. Neither can the various phases of the histology of the gall bladder such as the glands of Luschka (222) and the Rokitansky-Aschoff sinuses be considered (345). (For a more detailed consideration of the histology of the gall bladder, the reader is referred to the following references: (39) (92) (345).)

It is a matter of common knowledge that the gall bladder wall consists of four tunics, one of which is the tunica muscularis. The distribution and relative amount of the muscle fibers in the tunica muscularis vary in different species. As a general rule, the circularly arranged fibers predominate over those arranged longitudinally and obliquely. Whether the muscular tunic is thicker in the region of the neck of the gall bladder than in the fundus is not settled. Boyden (62) (70) indicates that the idea of the relative poverty of the gall bladder wall in muscle fibers was gained from microscopic studies of sections taken from the distended viscus and that sections taken from the viscus after evacuation in response to fatty foods present a different picture. For example, in the cat, passing from the distended to the empty state, the muscularis and serosa each increase ten times in thickness and the mucosa fifteen times. The muscularis of the biliary vesicle of the cat is as thick as the muscularis of the intestine of the guinea pig, but in the intestine, the fibers are packed much closer together (70). Abundant elastic tissue is present, and the serosa (cat) is nearly three times as thick as that of the intestine (62). From the physiologic viewpoint, the question is—does the gall bladder manifest the phenomenon of contractility, and if so, what is the maximal pressure exerted on its contents by its contractions?

Contractility. Direct observation. Galen (198), in his dissections, found the gall bladder sometimes very full, sometimes empty and sometimes in an intermediate state, and he taught by analogy that the gall bladder emptied like the other hollow viscera. Haller (221) and his students, in the study of irritability, found that the gall bladder of

different animals contracted after the local application of chemical irritants. In 1851, Dietrich, Gerlach and Hertz (150) reported that in an executed criminal they observed contraction of the gall bladder on electrical stimulation. Henle (241), in a similar experiment, obtained negative results.

A number of observers have reported that they have seen the gall bladder contract on being stimulated *electrically*. In the dog (84) (154) (164) (270) (318), a local shallow contraction may result, which has been recorded graphically by Doyon (154) and which may cause some evacuation (164). Some have failed to observe contractions (26) (220) (547). Adequate *mechanical* stimulation may cause a local shallow contraction (270) (547). Doyon (154) observed contraction on electrical stimulation of the gall bladder and bile ducts of the carp, adder, viper, rabbit and cat. In the guinea pig, contractions in response to electrical and mechanical stimulation are readily seen (101) (154) (248) (and others). The same is true for the opossum (162), in which complete evacuation may be obtained by repeated stimuli.

In addition to the above, many investigators have reported that by direct vision they have seen the gall bladder of man and of various animals contract after introducing procedures other than direct chemical, electrical or mechanical stimulation.

In dogs. Bainbridge and Dale (31), on the application of adrenalin, saw a marked contraction limited to about one-fifth of the gall bladder neck. Johnson (278), on the introduction of magnesium sulphate into the duodenum, observed a collapse of the fundus, followed by a minute wave of contraction which produced fulness of the cystic duct. Stepp and Duttmann (490), on introducing peptone into the duodenum, observed "ring contractions" which started in the fundus and passed toward the cystic duct, causing evacuation. Winkelstein and Aschner (548) injected pilocarpin intravenously (1/12 gr.) and observed the gall bladder wall tighten and decrease one-third in size. Mann and Higgins (365) stated that they have occasionally seen the gall bladder of cats and dogs contract and evacuate partially on stimulating the wall of the viscus immediately after death. Bruning (88) painted the gall bladder with barium chloride and saw a "tonic contraction" of the bladder with evacuation. (This is easily seen in the isolated whole gall bladder containing bile.) Lueth, Ivy and Kloster (342), after injecting cholecystokinin, observed a circular band of contraction which appeared and disappeared with the graphic pressure record which manifested a "tonus rhythm." They also observed during evacuation a longitudinal short-

ening, and a reduction in circumference and volume (a "tonic contraction"). Houssay and Rubio (256), in the "transplanted" or viviperfused gall bladder, obtained rises in pressure on introducing acid into the duodenum of the dog serving as a donor of blood. They did not see peristalses or local contractions. This is generally true of the dog's gall bladder. To see true "ring" or band contractions *in situ*, one must observe the gall bladders of a large number of dogs, unless he happens to be unusually fortunate. Houssay and Rubio (256) observed that if the gall bladder is cut, and then histamine or barium injected into the arterial supply, the contraction becomes visible. Others have failed to observe definite visual evidence of contraction of the gall bladder of the dog (26) (368) (and others).

In the cat. Boyden (70), on injecting adrenalin intravenously into the cat under local anesthesia, observed the gall bladder wall slowly pucker and withdraw itself from the margin of the fossa while decreasing in size.

In the guinea pig. Contractions of the gall bladder are very evident in the guinea pig. They have been described by Higgins (247) (248) (365) and by Burget and Brocklehurst (101), who have also described the anatomy of the biliary passages in this animal. Westphal (530) and Horsters (255) described peristaltoid movements of the gall bladder. Higgins and Mann (248) saw only localized areas of contraction (162a).

In the rabbit. Westphal (530) has described peristaltoid contractions of the gall bladder and common duct of the rabbit. Walsh (523) has confirmed this by injecting cholecystokinin, following which the contractions are sometimes as evident as they are in the guinea pig or fish.

In the fish. Higgins (245) has described the peristalsis and evacuation of the gall bladder of the bullhead (fish).

In birds. That the bile ducts of the pigeon, which is devoid of a gall bladder, contract spontaneously is a relatively old observation. Dujardin-Beaumetz (164) cites the literature up to 1873. The older observations were confirmed by Doyon (154) and by Copher and Illingworth (123).

In man. Bassler, Luckett and Lutz (36) were not able to observe at laparotomy a contraction of the human gall bladder on the instillation of magnesium sulphate into the duodenum. Sachs (462) saw evacuation without evident contraction, although in a personal communication to the author, he states that he believes it was not due to an aspiration process. Pribram (434) saw a "distinct contraction" associated with

evacuation, which was induced by instilling Witte's peptone solution into the duodenum. Venables and Knott (513) have quoted Doctor Haemerli as having seen vigorous contractions of the gall bladder after magnesium sulphate instillation. Matsuo (370) observed two cases under local anesthesia and on instillation of magnesium sulphate, saw each gall bladder contract and evacuate. Trommer and Hempel (508) introduced India ink into the gall bladder of patients with the abdomen open. On the instillation of 60 per cent glucose into the duodenum, they obtained evacuation in five cases without cystic duct obstruction. They did not look for evidence of contraction. Moser (398), in a case of hydrops with stones, on aspirating, observed the gall bladder "contract" so firmly as to outline the enclosed calculi. (See Haller (221).) Kalk (293) (294), using the method of *intra-abdominal endoscopy* (laparoscopy) and injecting pituitrin subcutaneoulsy, observed the gall bladder decrease gradually to one-half its original size, manifesting a "tonic contraction." He did not see sudden contractions or peristaltic waves.

Another very interesting type of evidence that indicates contraction of the gall bladder in man pertains to the movement of gall stones in the gall bladder after the introduction of various stimuli. Knox (310), Berg (40), Kalk and Schondube (300), and Dufour (163) have published exeellent x-ray plates showing a mass of stones grouped in the fundus before stimulation, and showing the stones "lined up" reaching from the fundus to the cystic duct after stimulation. The authors believed that the changes in the position of the stones was due to contraction. Indeed, it is difficult to interpret the x-ray plates in any other way.

Levine (330) also has observed the shifting of stones in the human gall bladder after a fat meal. He cited one case in which the stone was shifted from the fundus into the cystic duct (12) (153). In this connection, Whitaker (536) (537) placed a stone larger than the common duct into a cat's gall bladder and found it occluding the common duct after several days of fat feeding. Chauffard (109) has argued that the hypertrophy of the gall bladder muscle in cholelithiasis is an indirect proof of expulsive effort in man (92a).

Fluoroscopic observations. The fluoroscopic evidence on animals will not be reviewed. Only some of the fluoroscopic observations on the human in which the gall bladder was visualized by cholecystography, or x-ray plates made, will be mentioned, since the findings are not unlike those seen in animals. In these citations, various stimuli were used (pituitrin, magnesium sulphate, fat meal, etc.).

A broad tonic ring of contraction with decrease in size of the gall bladder was seen by some (113) (166) (303) (329) (330). Baetzner (30) reported observing "rhythmic movements of the fundus of the gall bladder." Kalk and Schondube (300) saw "contracting rings," but were not certain whether they were localized or peristaltoid. Whitaker (536) (537) has also observed "contraction rings." Pendergrass, Overholt and Ravdin (422) injected lipiodol into the gall bladder through a cholecystostomy, asked the patient to hold his breath, and saw what they thought were rhythmic contractions (512). Stewart and Illick (491) failed to observe evidence of contractile activity. Levine (330) has summarized his observations as follows: Different types of gall bladder contractions are seen, the most frequent type being slight, localized alterations in contour, which appear to be cyclic and to be accompanied by a tonic contraction of all the muscle fibers, which progressively increases until the vesicle has reached its smallest size.

Graphic records of motor activity of the gall bladder. The first graphic records of gall bladder motor activity were made by Doyon (154) in 1893. It is difficult for those acquainted with methods for studying visceral motor activity to believe that Doyon's records were due entirely to artifacts. However, Bainbridge and Dale (31) definitely established the contractility of the gall bladder in dogs by separating it from the liver and demonstrating at the same time that changes in liver volume may produce artificial changes in intra-gall bladder pressure (342) (501). Since then, graphic records demonstrating motor activity have been obtained by every investigator who has employed adequate methods and has taken cognizance of the sensitivity of the gall bladder in the dog to such factors as trauma, depth of anesthesia, blood pressure level, asphyxia, relation to meals and optimal pressure. Genuine contractions have been recorded from the gall bladder *in situ* in both anesthetized and unanesthetized animals, chiefly dogs, and from isolated "strips" and from the isolated whole gall bladder of guinea pigs, rabbits, cats, dogs, monkeys and man.

Types of motor activity. The gall bladder manifests two types of motor activity. One type may be called a *tonic contraction.* It produces a sustained rise in intra-gall bladder pressure which persists from five to thirty minutes or more. The other type may be called a *tonus rhythm*, which is manifested by a rhythmical contraction and relaxation occurring at a rate of from two to six times a minute. In each type, the musculature as a whole or a part of it may contract. In some species, as referred to above, a peristaltic, or peristaltoid type of motor activity during evacuation occurs.

The tonus rhythm. In the dog. The tonus rhythm of the gall bladder of the dog *in situ* has been recorded by numerous observers (31) (100) (154) (248) (256) (264) (342) (381) (419) (430) (501). It has also been observed in "strips" or in the isolated whole gall bladder of the dog (115) (116) (154) (228) (260) (336) (351) (442) (514). The tonus rhythm may be obtained from the gall bladder *in situ* in 60 per cent or more of dogs with adequate technique and may be modified by certain drugs and autacoids. The changes in pressure produced vary from 0.1 to 3.0 cm. of bile, and occasionally under stimulation to 4 or 5 cm. A subthreshold stimulus for a tonic contraction may augment the amplitude and frequency of the tonus rhythm. At the height of a tonic contraction, the tonus rhythm may disappear, to reappear (256) (264) in a gradually increasing amplitude. The tonus rhythm may be augmented by splanchnic nerve section (31). Increased tension may sometimes also augment the amplitude (31) (270) (419). The optimal pressure for observing the rhythm varies considerably, usually 5 to 7 cm. of bile. It may appear and disappear without apparent cause (419). Feeding increases the rate and amplitude (419) as does the introduction of digested fat into the intestine (264). A rise in temperature augments the tone and rhythm of the isolated gall bladder (115) (154) (256) (260), the optimal temperature being about 39°C.

In the guinea pig. A tonus rhythm in "strips" of, or in the isolated whole gall bladder of the guinea pig has been recorded by numerous investigators. They occur at a rate of from two to five per minute.

In the rabbit. Westphal (530) reports only a peristaltoid activity which occurs at a rate of from two to three per minute on appropriate stimulation. Doyon (154) was unable to record contractions in the rabbit because of the technical difficulties, which were apparently overcome by Westphal (530).

In the cat. Birch and Boyden (52), by studying x-ray plates in the cat, reported a tonus rhythm having a rate of from one and one-half to two minutes. In the strips of the cat's gall bladder, Macht (351) recorded a tonus rhythm, but he does not give the rate.

In the primates. In the isolated gall bladder of the monkey, Ravdin and Morrison (442) observed a rate of from two to six per minute. A diseased gall bladder removed from a patient showed an irregular contraction rhythm.

The tonic contraction. Pressure exerted. Although Doyon (154) and Bainbridge and Dale (31) recorded the pressure (10 to 15 cm.) produced by gall bladder contraction, Freese (192) was the first to attempt

to measure the force against which the gall bladder would contract. He recorded a pressure of from 21.4 to 31.3 cm. of Ringer's solution. Since then the maximum intra-gall bladder pressure has been measured under maximal stimulation in a number of animals by several observers.

In the dog. The maximum pressure recorded varies from 14.0 to 35 cm. of water (85) (100) (192) (248) (256) (264) (359) (364) (381) (419). For unanesthetized dogs, the maximal pressures reported post cibum vary from 24.0 to 26.0 cm. of bile (248) (264) (381). The resting intra-gall bladder pressure in unanesthetized dogs is around 10.0 cm. bile (248) (264) (381); in anesthetized dogs, it is quite variable—3.0 to 8.5 cm. water.

The maximum secretory pressure of bile in the dog ranges from 24.0 to 31.0 cm. water (237) (244) (355) (362) (381) (394) (407). In human patients, the secretory pressure of bile has been reported to vary from 21.0 to 27.0 cm. of bile (450a). It is of considerable teleologic interest that *the maximum power of gall bladder (normal) contraction is not greater than the secretory pressure of the liver.* Theoretically, it should not be otherwise, unless there were competent valves in the hepatic ducts.

In man. The pressure exerted by the contractions of the human gall bladder has not been measured.

In guinea pig, rabbit and opossum. Higgins and Mann (247) (365) found that the gall bladder of the guinea pig may contract against a pressure of from 7 to 8 cm. water. Burget and Brocklehurst (101) recorded a maximal pressure from the cystic duct during gall bladder contraction of 9.8 cm. water, the visible contractions causing rises of from 5 to 9 mm. pressure. In the rabbit, Westphal (530) has recorded pressures during contractions caused by vagus stimulation as high as 10 to 12.5 cm. of water. In the opossum, DuBois and Kistler (162) found pressures ranging from 1.5 to 4.0 cm. of bile in the cystic duct during contractions of the gall bladder.

Contraction elicited by the introduction of substances into the duodenum. Graphic records of increases in intra-gall bladder pressure have been made by numerous investigators after the introduction of various substances into the duodenum.

HCl. Bainbridge and Dale (31) introduced 0.4 per cent hydrochloric acid—quantity not stated—into the duodenum and failed to observe a rise in bladder pressure. However, Okada (419) obtained positive results in unanesthetized dogs on the introduction of 200 cc. of 0.4 per cent hydrochloric acid. This has been confirmed in anesthetized dogs, in which much smaller quantities of acid were used. Taylor and Wilson

(501) obtained an increase in tone of the "shielded" (protected from pressure of adjacent viscera) gall bladder with from 1 to 10 cc. of N/10 HCl. Ivy and Oldberg (264) obtained rises of from 3 to 6 cm. of bile pressure on introducing 15 to 40 cc. N/10 HCl. The latent period of response varies from two to five minutes. The contractions persist from ten to sixty minutes, usually depending on the amount of acid used.

Olive oil and fats. Okada (419) observed variable rises in intra-gall bladder pressure after the introduction of olive oil into the duodenum. Ivy and Oldberg (264), using anesthetized dogs, obtained no rise in pressure with fresh olive oil, cream and egg yolk. This was confirmed by Houssay and Rubio (256). They did obtain a gradually developing tonic contraction on which was superimposed an augmentation of the tonus rhythm with 1.5 per cent butyric acid, 5 per cent soap solution, butter, and pancreatic digests of olive oil, egg yolk and cream. The variation in the olive oil responses reported by Okada (419) may be due to the factor of rancidity, since Rost (456) has reported results indicating that the amount of bile formed and expelled into the duodenum in response to olive oil depends upon its rancidity. Higgins and Mann (248), after injecting egg yolk and cream into the duodenum, obtained pressures from the dog's gall bladder of from 21.0 to 26.0 cm. of bile, which they were unable to account for by adventitious factors (100) (381). In the fish (bullhead), Higgins (245) introduced egg yolk into the duodenum, with the abdomen open, and although he did not make pressure records, he observed after a latent period of one hour and fifteen minutes, first an increase in tonus and then active peristalsis and evacuation of the gall bladder and peristalsis of the common duct.

Magnesium sulphate. The only experiments in which actual records of intra-gall bladder pressure were made and in which rises were obtained after the introduction of magnesium sulphate (four injections of 40 cc. of 33 per cent magnesium sulphate) into the duodenum are those of Taylor and Wilson (501). The author and his colleagues have frequently introduced magnesium sulphate into the duodenum with negative results. If Taylor and Wilson had obtained their rises from the gall bladder by experiments in which liver volume changes were ruled out, their results would be unequivocal. However, their experiments were better designed to obtain reflex effects than ours, since we ligated the cystic duct. In order to obtain reflex effects, procedures must not be introduced which will interfere with or abolish the possible pathways, a fact that a number of investigators have overlooked on reporting negative results. From the data at hand, it may be stated that when placed in the duodenum,

magnesium sulphate does not act on the gall bladder by a humoral route, and that it is not unequivocally established that it acts reflexly to produce a gall bladder contraction (*vide infra*).

The action of drugs and other substances on the gall bladder will be reviewed later.

"*Postural tone.*" It has been pointed out above that an adequate or optimal intra-gall bladder pressure is required to obtain the best evidence of motor activity. The optimal pressures reported vary widely, apparently depending upon the experimental procedures employed and the tone of the gall bladder at any particular time. We never obtained a good contraction from dogs in the digestive state with a partially emptied or contracted gall bladder. Houssay and Rubio (256) found that after an alteration in pressure, the original pressure tends to return, which of course is true of all the hollow abdominal viscera (adaptive relaxation or contraction). The author can confirm that observation. Bainbridge and Dale (31) observed that distention augmented tonus rhythm, but did not cause a tonic contraction, which they thought might have been prevented from occurring by the anesthesia. The author has occasionally seen a contraction lasting for several minutes after a sudden moderate (5 to 6 cm. water pressure) distention. Hyperemia of the gall bladder augments tone (31), and anemia causes relaxation as a rule (31) (256). The effect of anemia is not due indirectly to vasoconstriction, since it produces relaxation after chrysotoxin (31). In the author's experience, the response of the gall bladder to anemia varies considerably. Occasionally in anesthetized dogs, the gall bladder may contract to a stimulus and remain contracted, without a change in liver volume or edema of the gall bladder, for one or two hours, relaxing only upon very deep ether or chloroform anesthesia.

Spontaneous tonic contractions. Tonic contractions of the gall bladder lasting from several minutes to an hour, apparently without experimental cause, are occasionally seen and have been recorded by a number of observers on the isolated gall bladder and the gall bladder *in situ*.

The records of pressure changes in the gall bladder are not always due to adventitious effects. That the pressure changes in the gall bladder resulting from certain stimuli are not due to pressure transmitted from the duodenum and stomach or abdominal wall has been conclusively demonstrated (342) (501) by recording simultaneously intra-gall bladder, intra-duodenal and intra-gastric pressures. An increase in liver volume such as occurs after the injection of hypotensive substances increases intra-gall bladder pressure (31) (342). The rise in intra-gall bladder

pressure resulting from an increase in liver volume in the dog may occasionally amount to as much as 8 cm. of bile pressure, depending upon the extent of venous engorgement of the liver and the extent to which the gall bladder is surrounded by the liver. As Bainbridge and Dale (31) first pointed out, this fact must be taken into consideration in interpreting the motor response of the gall bladder to various stimuli. Sometimes the pressure in the gall bladder continues to rise gradually from the initial level due to anomalous hepatic ducts which open directly into the gall bladder.

Contractions of the common bile duct. A peristaltic type of activity of the common bile duct in the pigeon has been pointed out above. Higgins (245) has described peristaltic-like contractions in the common duct of the fish (bullhead). In the opossum, DuBois and Hunt (160) saw peristaltic-like activity of the common duct which was followed by a contraction of the ampulla. In the opossum, the ampulla is located entirely outside the duodenal wall. Westphal (530) has reported peristalses (about four per minute) in the common duct of the guinea pig which begin in the mid-portion and proceed to the ampulla, which in turn contracts. Burget and Brocklehurst (101) have reported the same phenomenon in the common duct of the guinea pig, but believe it is passive, being produced by the suction of the ampulla. Westphal (530) reported the same phenomenon for rabbits and cats. Copher and Illingworth (123) placed iodized oil into the common duct of the cat which had been separated by ligature from the cystic and hepatic ducts. The iodized oil left the common duct in twelve hours. They thought this indicated common duct activity, but pointed out that the evacuation might have been due to the aspirating action of the duodenum.

Whitaker and Boyden (539) tied the cystic duct in the cat, placed a cannula in the common duct and observed that the hepatic bile flowed from the cannula with a conspicuous rhythm (ten minutes) which gradually decreased in intensity, but was revived again by an injection of pituitrin. They believed this rhythm was due to the muscle in the bile ducts and not to a secretory rhythm of the liver.

In the dog, Laborde (318) and Audige (25) found the common duct to contract on direct electrical stimulation. Doyon (154) observed contractions of the common duct of the dog on electrical stimulation of the splanchnic nerves. The contractions were indicated by movements of an oil level. Gerald (201) obtained rhythmic contractions from isolated strips of the common duct of sheep. Colin (119) observed contractions of the common duct of the horse.

Innervation of the gall bladder. Anatomy. The most recent study on the microscopic neuro-anatomy of the gall bladder is by Morowka (397). He describes a sympathetic plexus in the gall bladder wall containing ganglion cells. The muscle fibers are supplied by a relatively enormous number of exceedingly fine fibrils, which originate from the ganglion cells. There appears to be one neuro-fibril for each muscle fiber. Nerve fibrils also pass to the cells of the mucosa. Ischiyama (260) has described a similar intrinsic nerve plexus with ganglion cells.

Sensory innervation. Laborde (318) in 1874 obtained pain responses by injecting irritants into the biliary passages of dogs. Schrager and Ivy (472) studied the nervous pathways concerned in eliciting various responses induced by distention of the gall bladder and biliary passages. They found that pain, vomiting and respiratory disturbance resulted from distention. Pain was abolished by splanchnic section, chiefly the right; nausea and vomiting were abolished by vagi section; and the respiratory disturbances were reduced by section of either the splanchnics or vagi. Section of both vagi and splanchnics abolished all responses. This has been confirmed by Davis, Hart, Crain (142) and by the surgical use of spinal anesthesia in gall bladder and bile duct operations in man (395). Since distention of the gall bladder or biliary passage may induce pylorospasm (180), McEwen and Ivy (373) resected the pyloric antrum in dogs and found that the pain response to biliary tract distention persisted, indicating that pain from biliary tract distention is not due entirely to pylorospasm.

Motor innervation. The older literature has been reviewed by Mann (354). He concluded that the vagi and splanchnics carry both motor and inhibitory fibers, but the vagus is predominantly motor and the splanchnic inhibitory for the gall bladder. The more recent literature indicates that in the dog and cat vagus stimulation has little effect on the gall bladder (100) (132) (220) (536) (547). However, most of these studies have been directed towards ascertaining whether or not evacuation is induced by vagus stimulation. Whitaker (536) filled the gall bladder with iodized oil (dog and cat) and stimulated one vagus in the neck centrally and peripherally after recovery from anesthesia without observing evacuation, although the animals later evacuated to a fat meal. Employing the same method, Crandall (132) stimulated the vagus just above the diaphragm and failed to obtain evacuation, although evacuation was obtained with either a fat meal or cholecystokinin. These results, however, are not to be interpreted as indicating that the vagi and splanchnics have no effect on the gall bladder. The conclusion

of Mann still stands in the opinion of the author, the more recent results showing that electrical stimulation of the vagi does not lead to evacuation under the conditions of the experiments. Some contraction may have occurred without evacuation, since it is known that contraction of the gall bladder may occur without evacuation in the presence of a spastic sphincter of Oddi or duodenum. Westphal (530), using the guinea pig, made the following observations: stimulation of the vagus causes the gall bladder to contract. The response is augmented by previous splanchnic section. Whether or not evacuation results depends on the strength of the stimulus. A weak stimulus causes contraction of the gall bladder, relaxation of the sphincter, contraction of the ampulla and evacuation. A strong stimulus causes spasm of the gall bladder and ampulla, and evacuation does not result. This difference in response to vagus stimulation after weak and strong stimulation may explain some of the discrepancies in the literature. Section of the vagi is without much effect. Splanchnic stimulation causes relaxation of the gall bladder and ampulla and contraction of the sphincter papillae. After section of both vagi and splanchnics, the motility is normal. DuBois and Kistler, Burget and Brocklehurst (101) failed to obtain evidence of contractions of the gall bladder of the guinea pig on vagus stimulation. (See note, p. 102.)

Reciprocal innervation of the gall bladder and sphincter of Oddi. Heidenhain (237) attributed the first gush of bile after a meal to acid chyme passing over the papilla of Vater which caused a reflex contraction of the gall bladder. Presumably because of the rather sudden gush of dark bile that appeared sometimes after the application of dilute acid to the duodenum (Rutherford, Claude Bernard and others), Michael Foster (187) in 1880 wrote: "This, probably, is a reflex action leading to the contraction of the muscular walls of the gall bladder and ducts, accompanied by a relaxation of the sphincter of the orifice." One of the conclusions drawn by Doyon (154) from the results of his studies on the gall bladder and sphincter of Oddi was that "certain well-determined reflex excitations, such as excitation of the central end of the vagus nerves, provoke dilation of the duodenal sphincter parallel to the contraction of the gall bladder." Rost (455) from observations on dogs with a Pavlov biliary fistula, expressed a similar opinion. In 1917 Meltzer (385) discussed the "law of contrary innervation" (reciprocal innervation) as related to diseases of the gall bladder. He pointed out that during storage of bile, the sphincter is contracted and the gall bladder relaxed, and that during discharge, the gall bladder contracts and the sphincter relaxes. To his article, he appended a note suggesting the administration of 25

per cent magnesium sulphate solution by duodenal tube to relax the duodenal wall and sphincter which should permit the escape of bile in certain cases of jaundice and biliary colic. This suggestion was applied clinically by Lyon and developed into a procedure which has been termed "the non-surgical drainage of the gall bladder" (112) (347) (350). Credit is due Meltzer and Lyon for their provocative ideas which reawakened the interest of the medical world in gall bladder physiology—a topic that had disappeared from a number of textbooks of physiology in spite of its great practical importance.

A number of investigators (41) (99) (206) (276) (302) (536) (546) have attempted to obtain evidence showing that when the sphincter was relaxed, the gall bladder would contract. Some very obviously used wrongly devised technique to test the theory and obtained negative results.

The literature is quite generally agreed that magnesium sulphate introduced into the duodenum may decrease the resistance to the flow of bile into the intestine. Results indicating a "reciprocal innervation" have been reported by three groups of investigators. But magnesium sulphate was not the excitant used. Copher and Kodama (125), on the injection of adrenalin, observed a rise in gall badder pressure with a relaxation of the sphincter. Potter and Mann (430), on following intra-gall bladder and intra-common duct pressure simultaneously in unanesthetized dogs, observed "reciprocal pressure changes." McMaster and Elman (381), using a method called "triple intubation" (cystic duct, proximal and distal end of common duct cannulated and tubes leading externally), which appears to be an excellent method, obtained quite decisive results. On feeding or frequently on showing the animal food, the gall bladder pressure rose markedly and the sphincter resistance decreased. Although one may not consider this experiment crucial, in regard to the law of contrary innervation, it does appear that it is crucial in regard to a reciprocal response of the gall bladder and sphincter in response to a stimulus, which is essentially the conclusion of Doyon. One might think that the nervous pathways involved would be disturbed by the operative procedure, but they are not apparently. Kadokura and Katsuya (312) have repeated the work of McMaster and Elman, and have extended the method to include a duodenal fistula so that they could control the factor of ejection of chyme from the stomach. They found on feeding that the gall bladder contracted. The bile did not flow at once into the duodenum, but in intermittent portions as the chyme entered the duodenum. This was caused by the relaxation and

contraction of the sphincter. Their data confirmed essentially the findings of McMaster and Elman (381), except in that they emphasized the intermittent character of the relaxation of the sphincter.

Much indirect evidence might be pointed out in support of or against the theory, but this would not assist considerably in the solution of the problem. For example, Birch and Boyden (52), working with unanesthetized cats, sewed an electrode in place over the ampulla of Vater, cannulated the common duct, and filled the gall bladder with iodized oil. After initiating evacuation with egg yolk, they stimulated the ampulla. Evacuation ceased and the gall bladder became less elongated, or "relaxed." The most probable explanation of the phenomenon is that it was due to a reflex inhibition of the gall bladder. Whether the reflex started from the sphincter or duodenal muscle is open to question. If it was the ampulla that contracted, an intra-gall bladder pressure record would be necessary to show that the gall bladder was not forced to distend by the secretory pressure of liver. Cole (118) found that concentrated sodium bicarbonate solution irrigated through the stomach caused a spasm of the sphincter of Oddi or duodenum, and Boyden and Birch (52) observed that sodium bicarbonate solution intraduodenally caused an increase in size of the gall bladder shadow in man. Again, was the increase in the size of the shadow due to reciprocal relaxation of the gall bladder, or was it due to the passive distention of the gall bladder with the hepatic bile which could not pass the spastic sphincter? Egg yolk given subsequently by duodenal tube provoked a prompt evacuation. Did the egg yolk cause contraction and a reciprocal relaxation of the spastic sphincter, or did the egg yolk cause contraction of the gall bladder, which it unquestionably can do, and a direct relaxation of the sphincter? Only direct well controlled and devised experiments will settle such questions.

Evidence will be pointed out later which shows that a "reciprocal innervation" is not essential for gall bladder contraction and evacuation. It cannot be stated, though, that it has been demonstrated (42) (99) (202) that "reciprocal innervation" is not essential for "normal" evacuation, since in the animals used to show that reciprocal innervation is not essential for evacuation, the "normal" emptying time of the gall bladder was not established.

In regard to the question of reciprocal innervation, one should not be surprised at the disagreement in the literature because of the relatively slight changes in pressure to be recorded, the multiplicity of factors to be considered, and the variations in response of the sphincter from time to time. Even in the instance of the relation of the stomach to its sphinc-

ters, there is, or was disagreement, which is best explained by the fact that the muscular response of a sphincter is frequently determined by its neuro-muscular state at the time a stimulus is applied, i.e., if contracted, it is likely to relax, and if relaxed, it is likely to contract, to the same stimulus (104).

Action of drugs and autacoids on the gall bladder musculature. Pilocarpine. In dogs with the gall bladder *in situ*, an intravenous injection of 0.5 to 1 mgm. of pilocarpine will cause a rise in intra-gall bladder pressure (279) (516) (546) (547). Bainbridge and Dale (31), because they could not obtain this effect if the gall bladder were separated from the liver, thought the rise due to a swelling of the liver. However, if a record of liver volume is made, we find (516) that the rise in gall bladder pressure persists longer generally than the fall in blood pressure and increase in liver volume, and is immediately abolished by atropine. Eserine (100) and physostigmine (85) are reported to be without effect. That pilocarpine may cause gall bladder contraction in the dog is indicated also by the results of experiments on the isolated whole gall bladder or "strips" of the viscus. Pilocarpine (260) (514), eserine (114) (115), muscarine (260), and physostigmine (115) (260) (336) cause either a tonic contraction or an increase in tonus rhythm, usually the former. Pilocarpine causes an increase in intra-gall bladder pressure of the decerebrate cat and a contraction of the isolated gall bladder of the monkey (442). The gall bladder of the guinea pig *in situ* contracts and evacuates under the influence of pilocarpine, according to Westphal (530) and Du Bois and Kistler (162a); but Burget and Brocklehurst (101) attribute the evacuation to the activity of the duodenum. However, the isolated gall bladder of the guinea pig responds quickly and beautifully to pilocarpine (85) (176) (177) (291) (293) (442). Physostigmine (85) is said to be without effect.

Atropine causes varying degrees of relaxation of the gall bladder *in situ*, or isolated, in all species studied,—guinea pig, cat, dog, monkey,—and antagonizes the action of pilocarpine and choline (31) (85) (100) (114) (115) (176) (177) (228) (260) (264) (291) (336) (342) (351) (442) (514) (530) (546) (547).

Choline (acetyl choline) causes the isolated gall bladder (dogs and guinea pigs) to contract quickly and markedly (85) (260) (291). Its effect is antagonized by atropine. In biliary fistula dogs, Wood (552) observed a flow of dark bile after the injection of choline, but when he placed a balloon into the gall bladder, he failed to obtain evidence of contraction. Voegtlin and Ivy (516) have obtained no evidence of contraction in the dog's gall bladder *in situ* on injecting choline or acetyl choline, the change

in liver volume explaining the changes observed. If it causes a contraction of the gall bladder *in situ* in the dog, the effect is masked by the change in the liver volume.

Ischiyama (260) isolated a choline-like substance from the gall bladder mucosa which caused an increase in tone of the isolated gall bladder. This has been referred to as the "vesicular hormone"!

Epinephrine in small doses is variable in its action on the isolated or gall bladder *in situ* (31) (85) (100) (115) (176) (228) (260) (293) (322) (336) (442) (501) (530) (546) (547). Some report no effect, many relaxation, and others a transient contraction. In larger doses (1 mgm. down to 1 cc. to 6 cc. of 1:50,000), it may cause a diphasic (31) or a triphasic response (501) of the dog's gall bladder *in situ*. Westphal (530), (rabbit—gall bladder *in situ*), who observed epinephrine to cause only relaxation, thought that after pilocarpine, the epinephrine action was reversed. He did not perform enough experiments, however, to rule out a chance contraction. Bainbridge and Dale (31) saw a marked contraction occur in the dog's gall bladder *in situ* after direct application of a 1:1000 solution. Kalk (293) reported that the contraction of the isolated guinea pig's gall bladder caused by pilocarpine is relaxed uniformly by epinephrine.

Nicotine relaxes the gall bladder *in situ* (31). In the isolated gall bladder of the dog, nicotine (1/15,000) is reported to increase the tonus slightly (260).

Ergotamine causes a slight increase in intra-gall bladder (*in situ*) pressure of dogs and may increase the tonus rhythm (31) (85) (342). In the isolated guinea pig's gall bladder, contraction may result (85) (293). Erbsen and Damm (176) report no effect.

Histamine in any dose causes a rise in intra-gall bladder pressure in the dog's gall bladder *in situ* (85) (342). The chief factor concerned is the increase in liver volume. If histamine acts at all on the dog's gall bladder *in situ* in usual doses, its effect is entirely masked by the liver volume change (342). In the cat, histamine has little or no effect on intra-gall bladder pressure (264). Histamine (1:125,000) causes a marked contraction of the isolated gall bladder of the dog and guinea pig (85) (176) (177) (228) (255) (293) (442). Erbsen (177) reports that histamine causes the distended isolated gall bladder to contract, but has no effect on the empty gall bladder. Horsters (255) uses the isolated gall bladder of the guinea pig filled with bile to demonstrate contraction and evacuation, the gall bladder contracting and evacuating on the application of histamine to its serosa.

Pituitrin, according to some reports, either relaxes or has no effect on the gall bladder *in situ*, or isolated, of dogs and guinea pigs (85) (228). According to Erbsen (176) (293), the empty gall bladder is either not affected or is relaxed, but the full gall bladder contracts after pituitrin (293). Houssay (256) reports that the dog's gall bladder contracts after pituitrin. Kalk (293) observes the intravenous injection of pituitrin in the decerebrate cat causes a primary relaxation which is followed by a secondary rise in gall bladder tone after from five to fifteen minutes.

Morphine augments the amplitude of the tonus rhythm of the gall bladder *in situ* of dogs (342) (419) and rabbits (530). In the cat, Westphal (530) reports relaxation. Taylor and Wilson (501) in the dog found no effect other than that morphine tended to eliminate the relaxation caused by epinephrine. Lieb and McWhorter (336), in the isolated gall bladder of dogs, obtained, as a rule, no effect with therapeutic doses of morphine, but sometimes observed an augmentation of the tonus rhythm. Macht (351) found that methyl and di-methyl morphine and codeine either had no effect or increased the tonus of gall bladder strips (cats, dogs, pigs). Papaverine particularly, and narcotine, narcine and pantopon tended to decrease tonus. Erbsen (177) and Erbsen and Damm (176), although finding (guinea pig's isolated gall bladder) that cocaine and novocaine decreased tonus, confirmed Macht in regard to papaverine. In regard to morphine, the results of Lieb and McWhorter have been confirmed (177) (351). Erbsen (177), as is well known for the gall bladder *in situ*, found that ether decreased the tonus of the isolated gall bladder.

The nitrites relax the gall bladder of the dog *in situ* and isolated "strips" (177). Chloral hydrate induces a regular tonus rhythm with an augmented amplitude in unanesthetized dogs (419). Guanidine has no effect on the isolated gall bladder of the guinea pig (176).

Strophanthin (336) increases the tonus and augments the tonus rhythm of gall bladder "strips" from dogs. Yohimbine (256) augments the tonus rhythm but causes slight relaxation.

Barium chloride causes a contraction of the gall bladder both *in situ* and isolated (88) (100) (176) (260) (442) (and others). Magnesium sulphate intravenously tends to relax the gall bladder of dogs (342) *in situ*, as well as the isolated gall bladder of the guinea pig (85) (176). Intravenous hypertonic sodium chloride solution (8 to 12 cc. of 30 per cent) is inconstant in its action on the gall bladder *in situ* of dogs (501) (517). Erbsen and Damm (176) (177) report that the potassium ion causes contraction, and the calcium ion relaxation of the isolated guinea pig's gall bladder.

Thyroxin (176) and insulin (123) (176) have no effect on the isolated gall bladder of the guinea pig.

Secretin has been injected intravenously by a number of workers, to determine if the gall bladder might be caused to contract, with negative results (123) (418) (501) (536) (546), probably because the method of preparation and the doses used were inadequate. Brago and Campos (78) in 1919, on injecting secretin into a biliary-duodenal fistula dog, concluded that because of the character and quantity of bile poured out through the fistula, a contraction of the gall bladder must have occurred. Brugsch and Horsters (85) injected secretin and obtained as much as 11 cm. of water rise in intra-gall bladder pressure and on succeeding injections as much as 28 cm. The individual contractions lasted about fifteen minutes. None of these secretin preparations was free of vasodilatin, although some were relatively so. Ivy and Oldberg (264) obtained contractions (graphic record, gall bladder *in situ*) on the intravenous injection into cats and dogs of a vasodilatin-free secretin preparation, the active principle of which later proved to be a substance (cholecystokinin) different from, but probably closely related chemically to, secretin. This has been confirmed on dogs by Still (493) and Houssay and Rubio (256).

From the isolated gall bladder, or strips, contractions have been obtained on the introduction of secretin into the bath (85) (260) (384) (442). Kalk and Nissen (297) obtained contractions from "spinach secretin;" but one cannot be certain that the extracts used in these experiments were free of histamine. They very likely contained considerable histamine. Halpert and Lewis (228) used a preparation of cholecystokinin on the isolated dog's gall bladder and could not obtain a contraction, although their gall bladder preparation responded to histamine and pilocarpine. Jung and Greengard (291), using a highly concentrated cholecystokinin preparation known to be free of vasodilatin, obtained contractions of the isolated gall bladder of the guinea pig, which were not affected by atropine, and which were not due to errors in pH and other defects incident to so much isolated smooth muscle investigation in the past. Inactivated cholescystokinin solution was ineffective. They found that about fifty times the amount to cause a contraction of the gall bladder (*in situ*) of the barbitalized dog was required. They ascribed the large dosage requirement to the large molecular size and relatively low diffusibility of cholecystokinin.

The pharmacologic studies of Houssay and Rubio (257) on the "transplanted" or viviperfused gall bladder deserve special mention. The

method is free from a number of objections that may be raised against the pharmacologic study of the organ *in situ* in the abdomen and the use of "strips," or the isolated whole gall bladder. Their work, which was very extensive and thorough, may be briefly summarized as follows: Sympathico-mimetic drugs, especially epinephrine, relax the gall bladder. Veratrine and ephedrine are less effective. These drugs cause relaxation after atropine or yohimbine. Very slight relaxation may still be obtained after ergotamine. Parasympathico-mimetic drugs cause contraction (acetyl choline, pilocarpine), atropine abolishing their effect. Quinine causes a slight contraction. Nicotine, alpha-lobeline, muscarine are amphotropic, and after atropine, cause relaxation only. Histamine, barium chloride, "pitocin," the cyanides, peptone and calcium choride cause contraction, the first two being the most active. Papaverine, magnesium chloride, phenol and sodium salicylate cause relaxation, but considerably less than the sympathico-mimetic drugs. Bile salts, caffeine, cocaine, beta-tetrahydronaphthylamine, morphine, chloralose, urotropine, potassium chloride and sodium citrate were ineffective in the doses employed.

EVACUATION OF THE GALL BLADDER. Opinion has not been unanimous in regard to the function of the motor activity of the gall bladder. Some have maintained that the motor activity serves the sole purpose of facilitating absorption (54) (55) (147) (225) (497). Others have maintained that the gall bladder musculature serves to prevent distention. Most investigators now maintain that the musculature functions to expel bile. By analogy with the intestine, it probably performs all three functions.

Evacuation in man. Three types of evidence unequivocally demonstrate that evacuation of the gall bladder occurs in man via the cystic duct, namely, surgical, chemical analysis of duodenal contents and roentgenological.

The surgical evidence showing evacuation and contraction of the human gall bladder during laparotomy and laparoscopy has been outlined briefly above under the subject of contraction.

The chemical evidence showing that the gall bladder of man may evacuate has been obtained by analyzing the duodenal drainage fluid before and after the introduction of substances expected to cause gall bladder contraction and evacuation. Chemical analyses for iodine, for pigment and cholesterol have been made. The analyses of duodenal contents for iodine were made after the gall bladder had been visualized with tetraiodophenolphthalein. In order to make the following data clear, it should be pointed out that the first yellow duodenal fluid is

called "A" bile, the dark bile obtained after the introduction of the stimulus is called "B" bile, and the subsequent drainage is called "C" bile. Feissly (179) obtained thirteen times more iodine from B bile than from A bile and three times more than from C bile. Fitts (181) found the iodine in B bile eleven times more concentrated than in A bile. Lake (320) found that B bile contained forty-eight times as much iodine as A bile and ten and eight-tenths times as much as C bile. Lyon (348) reported analogous results. In these studies, gall bladder evacuation was caused by the introduction of magnesium sulphate or olive oil into the duodenum. These results on the human are similar to the findings reported by Mann and Higgins (248) for the dog. Sweet (499) [see Blond (54) (55)] has interpreted these results as indicating that the stimulus increases the absorption of iodine by the gall bladder which is then reëxcreted by the liver into the bile. Such a view is not tenable in view of the rapid evacuation or disappearance of the shadow (twenty to thirty minutes) in some subjects and the relatively slow rate of absorption indicated by the evidence cited above on the absorption of tetraiodophenolphthalein from the gall bladder. *Numerous investigators have analyzed the duodenal contents for bile pigment and cholesterol.* The reported results are variable not only because of the dilution factors (pancreatic juice, hepatic bile and gastric contents), but also because the amount of evacuation obtained varies quite widely. The most convincing results the author has been able to find are those of Kalk and Schondube (300). They stimulated the gall bladder with pituitrin and observed increases in bilirubin concentration in the duodenal fluid up to as high as 850 mgm. per cent. In one case they obtained B bile after pituitrin with a bilirubin concentration of 816 mgm. per cent, and then at operation obtained gall bladder bile that assayed 850 mgm. per cent bilirubin. Analogous results were also obtained in a second case. Other results, which are not so convincing, but in which the bilirubin concentration is increased to such an extent that the only place from which it could come in normal individuals is the gall bladder, are those of Adlersberg and Taubenhaus (5) (6) (7) (8), Jones (284), Kalk (293), and Rosenthal and Zinner (453).

The cholecystographic evidence showing that the human gall bladder evacuates through the cystic duct has accumulated rapidly in recent years and is very convincing. The rapid changes which occur in size and contour of the gall bladder within two to fifteen minutes after the ingestion of a fat meal, or the use of other procedures, as demonstrated by Boyden (69) (70) (against gravity, subject standing) and others (106) (269) (485)—there is an extensive literature—cannot rationally be inter-

preted otherwise. The most interesting pictorial evidence showing that the human gall bladder evacuates via the cystic duct is that the cystic and common bile ducts, the hepatic ducts occasionally, become visualized after the ingestion of egg yolk and cream, sometimes within ten or twenty minutes. The author (261) has seen numerous such films on the service of Dr. James T. Case, who observes this phenomenon in about 10 per cent of patients with normal cholecystograms. The author has found fourteen similar reports in the literature (3) (30) (38) (69) (70) (75) (206) (281) (328) (330) (410) (422) (468) (491). This, of course, has been observed in the cat and dog by every investigator using iodized oil. Boyden and Fuller (75) found that they were able to visualize the cystic and common duct in three-fourths of twenty-eight boys and girls. Such evidence conclusively demonstrates that the human gall bladder may evacuate via the cystic duct and is as absolute as the iodized oil cholecystographic evidence obtained in animals.

Evacuation of dyes and non-absorbable materials from the gall bladder. The literature on the evacuation of dyes and India ink introduced directly into the gall bladder of man has been reviewed above. In addition, Tada and Nakashima (500) injected AZO rubin S or soluble indigocarmine intravenously with a duodenal tube in place. After five hours, the dye no longer appeared in the duodenum. Some time later, magnesium sulphate was introduced into the duodenum and much dye was obtained. By puncturing the gall bladder at operation in man, it was found that the source of the stored dye was the gall bladder. These observations have been confirmed under somewhat modified procedures (229) (293). Evidence of evacuation in the dog has been obtained by using methylene blue somewhat similarly (446).

The evacuation of various substances (dyes of all kinds, various radiological opaque solutions, sand and gall stones) from the gall bladder of dogs, cats and rabbits is recorded in the following references (2) (12) (26) (61) (67) (91) (144) (149) (278) (302) (316a) (429) (461) (490) (523) (536) (537) (542).

Factors inducing or modifying evacuation. Psychic factors. Perception of food. In dogs, on the perception of food, McMaster and Elman (381) observed expulsion of bile from the gall bladder and relaxation of the sphincter of Oddi. Working with biliary fistula dogs, Bruno (89) observed a psychic discharge of bile. Puestow (436) observed it only sometimes. Crandall (132) "sham fed" (esophagostomy and gastric fistula) four dogs in which the gall bladder had been visualized. He failed to observe any evacuation. He removed the gastric juice result-

ing from "sham" feeding in order to avoid the possibility of it causing evacuation of the gall bladder on escaping into the duodenum. Krause and Whitaker (315) failed to observe evacuation in fasted cats on the perception of food. *In man*, the observations are also contradictory. Boyden (66) at first failed to obtain evacuation on the perception of food which was also the experience of others (132) (485). Later, however, (69) (76) Boyden reported that a slight evacuation (average 6 cc.) may be obtained in some human subjects on the presentation of some such savory food as crisp fried bacon. Occasionally, however, the gall bladder shadow may even increase. Such discrepancies indicate that the response is slight and variable.

Fear, anxiety and pain. In cats, Boyden (61) made observations which indicated that emotional factors caused some evacuation, which at first he was inclined to attribute to a discharge of epinephrine. After removal of the adrenals, he observed a response, but to a lesser degree, which he interpreted as due to direct nervous stimulation of the gall bladder. Whitaker (536), on repeating the experiments, obtained variable results. Puestow (436), working with an excellent biliary fistula preparation, observed that excitement sometimes delayed or inhibited the discharge of bile. Johnson (278) found that while duodenal drainage was being conducted in dogs, fright caused the drainage to cease. Sleep promoted drainage. He ascribed these effects to changes in the tone of the sphincter. Westphal (531) has emphasized the possibility that anxiety, or neurological factors, may play an important rôle in delaying evacuation of the gall bladder in man, either by affecting the gall bladder, or the sphincter, or both. This subject may be elucidated only by very careful studies in which the method of Elman and McMaster (381) of "triple intubation" is employed, simultaneously recording duodenal pressure changes (312).

Foods.[1] Boyden's (60) discovery that egg yolk and cream would frequently completely evacuate the gall bladder of the cat stimulated studies on the effect of various food substances. The literature shows undoubtedly that fat and fatty acids are the most active evacuants, proteins (meat) rank next, and carbohydrates last (60) (61) (62) (315) (132), the latter being comparatively ineffective in causing gall bladder evacuation. Boyden (61) (69) reports that more complete evacuation in man, as well as the cat, is obtained with egg yolk than with other fats. All

[1] Only cholecystographic evidence or evidence from duodenal drainage experiments in which bilirubin determinations showed an increase in concentration of pigment above that normally found in hepatic bile is considered.

fats are about equal in causing the initial phase of emptying. Five egg yolks are most effective; one is sufficient, however, to cause considerable initial evacuation. Small amounts of egg yolk, introduced at intervals into the duodenum, cause progressive diminution of the gall bladder shadow. Numerous fats have been found to cause evacuation when given by mouth or by duodenal tube; egg yolk (60) (61) (233) (293) (315) (and many others), olive oil (293) (303) (315) (320) (348) (390) (468) (490), oleic acid (125) (315) (331) (337) (371), cod liver oil (315), butter (69) (315), cocoanut oil (315), palmitic acid (315), peanut oil (216), castor oil (216), lard (60) (62), and cream and milk (60) (69) (315). Boyden (60) (61) (69) reports that the lecithin fraction of egg yolk is the most potent fraction, but pure lecithin is not as efficacious as the crude. Hard boiled yolks are said not to be as effective as raw yolks (61). Olive oil is more effective than magnesium sulphate (74) (215) or pituitrin (293) (390) (subcutaneously).

Liquid petrolatum. Liquid petrolatum does not evacuate the gall bladder (74) (216). Boyden and Birch (74) report that in two cases it decreased the efficiency of egg yolk subsequently ingested; but after the ingestion of a "coarse" full meal, egg yolk was again effective.

Egg yolk acting only in the stomach. Whitaker (536) obstructed the pylorus in two cats and found that on feeding egg yolk, the gall bladder did not evacuate. Morgan, Crandall and Ivy (396) perfused the stomachs of five dogs with a pouch of the entire stomach, the gall bladder having been visualized previously with the Graham-Cole technique. They observed no emptying in three dogs and emptying in two. Hence, evacuation of the gall bladder may be initiated from the stomach occasionally. Kalk (296) has reported that the gall bladder visualized in a patient with a complete pyloric stenosis, but on ingesting egg yolk, evacuation did not result.

Peptones. Of the whole protein foods, meat appears to be the best for promoting evacuation (61) (62) (315). Egg white (61) (62) (315) and casein (315) have but a slight effect, if any. Peptone (Witte's) is quite effective (252) (303) (308) (315) (434) (435) (455) (490), and according to some reports is as effective as fats; according to others, it is not as effective (168) (348) (485).

Water. Hamrich (233) found in dogs, in which the gall bladder was filled with iodized oil, that the drinking of water did not cause evacuation. (The author has confirmed this.) However, in eleven out of fourteen human subjects, Boyden (69) observed an average decrease in the gall bladder shadow amounting to 5.5 cc. after drinking a glass of water

(76). Chiray and Pavel (116) report that warm water intra-duodenally causes some evacuation.

Dilute HCl. That the injection of dilute hydrochloric acid into the duodenum will cause the gall bladder in dogs to contract has been pointed out above. Elman and McMaster (172) reported that the introduction of acid into the stomach of dogs may also relax the sphincter of Oddi (118) (278) (547) (548). Its effect in producing evacuation, however, is variable, as might be expected in view of the possibility that the sensitivity of the duodenum or sphincter to acid may vary. In man, Chiray and Pavel (116) reported evacuation on introducing warm hydrochloric acid into the duodenum. Boyden and Birch (74) and Whitaker (35) (537) obtained variable results. In the author's teaching laboratory, we have used dilute hydrochloric acid (20 to 50 cc. N/20 HCl) and magnesium sulphate in routine demonstrations and have found that variable results are obtained, the latter being much more likely to yield "B" bile, or bile high in pigment and cholesterol concentration.

Is digestion of fat necessary? Both Whitaker and Boyden have expressed the opinion that some digestion of fat must proceed before much, or complete, evacuation is obtained. This would appear to be the case from the observation of Ivy and Oldberg (264) that only digested fat or fatty foods containing considerable fatty acid, provoked a gall bladder contraction when introduced into the duodenum of anesthetized dogs. Copher and Illingworth (123) and Whitaker (536) have separated the pancreas from the duodenum in cats and dogs. The former observers obtained no evacuation, but the latter did. Silverman (477) observed evacuation with egg yolk in dogs with ligated pancreatic ducts. There is no doubt that evacuation will occur in animals which have been deprived of the pancreatic juice and are in good condition. One should expect considerable variation because the intestinal digestion of some animals is better after deprivation of pancreatic juice than others. Good digestion of the fat should speed the rate of evacuation.

Bile and bile salts. Bainbridge and Dale (31) and Taylor and Wilson (501) found that the intravenous injection of small quantities of bile decreased the tone and depressed the tonus rhythm of the gall bladder. This has been confirmed for both bile and bile salts (264) (342). Bile salts cause gall bladder "strips" (dog) to relax (336). Thus, it is not surprising that definite evidence of evacuation of the gall bladder in man or animals has not been observed on the administration of bile salts by mouth or intravenously (35) (179) (486) (536). However, as Graham

(211) has pointed out, it is likely that an increase in bile formation produced by chologenetic substances (bile salts and neocincophen) may cause more rapid disappearance of the cholecystographic shadow than would otherwise occur in the fasting state when bile formation is at a low ebb. Sufficient evidence is not at hand to warrant a definite statement, although Adler (3) reports that dehydrocholic acid given intravenously first causes distention of the gall bladder, followed by some disappearance of the shadow without an apparent contraction.

Effect of mechanical distention of the duodenum and stomach on evacuation. Boyden (69) (77) observed a variable amount of evacuation of the visualized gall bladder in three of four human subjects after the injection of air into the duodenum. The response was small when compared to the effect of the subsequent ingestion of egg yolk. Ladislaus (319) obtained no evidence of evacuation. Hamrick (233) obtained slight emptying of the gall bladder in dogs on distending the stomach.

Introduction of egg yolk at various levels of intestine on evacuation. Bronner and Schuller (83) found by experiments on man and cats that the introduction of egg yolk into the ileum, colon and rectum did not cause evacuation of the gall bladder.

Evacuation after various operations on the stomach. In gastrectomized dogs (396), the gall bladder visualizes and evacuates in response to egg yolk ingestion. The gall bladder of the gastrectomized cat also evacuates to egg yolk (538).

Patients and dogs with gastro-jejunostomy visualize and evacuate (298) (370a) (431) (462a) (485) (536). The gall bladders of dogs in which the stomach is anastomosed to the jejunum and the duodenal secretion drained into the lower ileum (Mann-Williamson, or Exalto, operation) visualize and evacuate to egg yolk (431). These facts show that it is not necessary for the egg yolk and cream to pass through the duodenum in order to produce gall bladder evacuation.

Copher and Illingworth (123) observed evacuation of the gall bladder after a fat meal in animals in which the appendix, spleen and adrenals had been removed, and in which acute appendicitis had been produced.

Evacuation produced by saline cathartics, chiefly magnesium sulphate. The literature bearing on the question of evacuation of the dog's gall bladder after the introduction of magnesium sulphate (25 or 33 per cent solution in 20 or 50 cc. amounts) is discordant. In anesthetized dogs, positive results have been been obtained by some (439) (490) and negative results by others (26) (149) (220). In unanesthetized dogs, varying degrees of evacuation of gall bladder bile or of dyes has been reported by

all observers (87) (278) (547) (548). On using various types of duodenal fistula dogs, evidence of evacuation has not been obtained (191) (205) (302). The negative results reported may have been due to adverse experimental conditions. Space does not permit a critical analysis of the experiments. It should be pointed out, however, that the effect of intraduodenal magnesium sulphate has not been studied on the dog's gall bladder visualized with tetraiodophenolphthalein. In the cat, however, a study has been made. Magnesium sulphate instillation was ineffective (35) (74).

It is established that concentrated magnesium sulphate introduced intra-duodenally will generally cause some evacuation of the gall bladder in the normal human subject. This statement is based only on the literature in which cholecystographic evidence is reported, or in which adequate chemical analysis of the drainage fluid was done.

Soon after Lyon published his paper on non-surgical drainage of the gall bladder in man, Dunn and Connell (165) and Bassler, Luckett and Lutz (36) obtained dark or "B" bile from a cholecystectomized patient. Lyon, Bartle and Ellison (349) also reported a case. However, Lyon (350) failed to obtain dark, or "B" bile from seventy other cases of cholecystectomy. This has been confirmed by several other reports (136) (137) (284) (490). In this connection, it should be borne in mind that if a portion of the gall bladder is left behind at operation, it may dilate and hypertrophy to a considerable size (*vide infra*). It is also possible in some cases that some concentration of pigment may occur in a dilated common duct. Or more likely, dark bile may be obtained as a result of the change of bilirubin to biliverdin in the dilated ducts (see Rous and McMaster (456) (457)).

The author has failed to find in the extensive "magnesium sulphate" literature a completely negative report insofar as evacuation of the gall bladder is concerned, that is, a report in which a fairly large series of normal subjects was used, and in which some evidence of evacuation was not obtained in some of the subjects in the series studied. The story is different, however, in regard to therapeutic and diagnostic efficacy. (For the evidence on pigment or dye elimination, note the following references and those cited before (5) (110) (112) (136) (137) (196) (229) (284) (319) (349) (350) (371) (490). For evidence on decrease or disappearance of the cholecystographic shadow, note the following references and those cited before (45) (74) (168) (179) (181) (215) (320) (348) (479) (485).)

The consensus of opinion is that magnesium sulphate is not as effective

as egg yolk and cream or as olive oil or oleic acid. In effectiveness, Boyden and Birch (74) compare the usual dose of magnesium sulphate favorably to the initial phase of egg yolk evacuation (from two-thirds to three-fourths evacuation in an average time of twenty-six minutes).

Soper (484) claims that magnesium sulphate is as effective by mouth as intra-duodenally. Meltzer (385) states that it is not as effective in promoting relaxation of the duodenum, and Bernstein and Held (45) believe that it is not as effective. Boyden and Birch (74) believe that it is almost as effective when taken by mouth. Before this question can be answered definitely, more cholecystographic evidence than is now available must be produced. Most of the advocates of the "non-surgical drainage of the gall bladder" insist that the optimal position of the duodenal sound is the proximity of the ampulla of Vater, and Friedrich (196) presents some evidence in suport of this contention.

Boyden and Birch (74) have made a careful, but not extensive, cholecystographic study in normal human subjects of the effect of various saline cathartics on the cholecystographic shadow. They found that magnesium sulphate, magnesium chloride, and sodium sulphate intraduodenally are almost equally effective in evacuating the gall bladder. They cause usually about two-thirds evacuation. Magnesium sulphate appeared to be the most potent. Sodium tartrate was only slightly effective and hypertonic sodium chloride, bicarbonate (485), phosphate and citrate caused filling or relaxation of the gall bladder instead of emptying. These authors discuss the possible theories on how magnesium sulphate acts, and although favoring the theory of a reciprocal reflex mechanism, rightly state, in the opinion of the author, that insufficient evidence is at hand to answer the question satisfactorily.

Evacuation and sex differences. Mann and Higgins (365) have studied sex differences in the degree of evacuation in dogs, guinea pigs and gophers. They found no difference except when pregnancy was present (*vide infra*). Boyden (69) has studied twenty-four normal human subjects of the same age group (twelve males and twelve females). His data showed that it takes the gall bladder of the male about twice as long as that of the female to reduce its volume to an arbitrary content of 3 cc. He thought this sex difference might be due to a difference in gastro-intestinal motility, but the results of studies on this point were negative.

Evacuation and pregnancy. Westphal (530) made observations which led him to believe that the tone of the sphincter of Oddi is increased in pregnancy. Kalk and Schondube (300) have reported that the gall bladder of the pregnant woman contracts earlier and more quickly after

a subcutaneous injection of pituitrin than that of the non-pregnant woman. Levyn, Beck and Aaron (333) found that in seventeen cases of primiparae, the gall bladder evacuated normally to egg yolk and cream (537). Fogelson (185) confirmed this report, but he found in addition that four patients who showed good shadows at the fourth or fifth month, failed to visualize at the seventh and eighth months (138). Crossen and Moore (135) obtained normal shadows in only three of twenty-two pregnant women in the fortieth week. They attributed most of the variations from the normal to technical roentgenological difficulties.

Mann and Higgins (367) observed that in pregnant dogs, guinea pigs and gophers (striped), the gall bladder either does not empty, or empties only partially, with the exception that the gall bladder of some pregnant dogs empties quite normally. They suggested that this might be due to disturbed gastro-intestinal motility. In pregnant cats, Whitaker and Emerson (540) obtained almost complete emptying. Further investigation of this very important and practical problem should be undertaken. It is believed that Boyden's "quantitative method" should be employed in order to obtain the most reliable results.

Evacuation in children. The observation that the gall bladder of children will visualize and evacuate is of special interest, since it has been claimed (226) that the Heisterian valves are more competent in children. Boyden and Birch (73) were unable to obtain visualization in babies by giving an emulsion of tetraiodophenolphthalein (probably too irritating and caused diarrhea). But in three children ranging from five to nine years, visualization and evacuation to egg yolk was obtained. More recently Boyden and Fuller (75) have reported observations on sixteen boys and twelve girls between the ages of six and eleven years. About fifteen cholecystograms were made on each subject. Their results indicated that the gall bladder of children evacuates faster than that of students of college age. On further study of the data on adults and children, it was found that the rate of evacuation of the gall bladder of the male slows down after puberty, while that of the female remains unchanged (75a). Their preliminary results indicate that the gall bladder content of children is proportionately greater in volume than that of adults.

Evacuation and habitus. It is apparently agreed that habitus and position of the gall bladder are related. However, Graham ((211), page 280), who has reviewed the literature, believes that a correlation between habitus and the degree and rate of evacuation has yet to be demonstrated. Boyden (69) whose data permit a more accurate statement, has reaffirmed this view.

Vitamines. Russell and Chichester (459) have reported that chickens fed a vitamin D deficient diet on autopsy have gall bladders larger and more distended than normal controls. This observation may be of interest in regard to the reports that small calculi may be found in the biliary tract of rats on a vitamin A deficient diet (313) (403) (511), and also in regard to the report that bile plays an important rôle in sterol absorption (93) (471).

Evacuation as influenced by drugs and autacoids. Pituitrin. The literature on the evacuation of the gall bladder of man after a subcutaneous injection of pituitrin (1 or 2 cc.) is extensive (3) (5) (8) (35) (43) (45) (61) (111) (166) (178) (179) (184) (252) (293) (295) (299) (300) (303) (390) (433) (435) (453) (464) (468) (466) (485) (539). Evacuation after pituitrin has been demonstrated by a decrease in the cholecystographic shadow or by analysis of the duodenal contents. In regard to the effectiveness of pituitrin, the reports vary. Most observers agree with Kalk (293) (294), the originator of the "pituitrin gall bladder test," that it is not as effective as olive oil or egg yolk. Kalk, however, thinks that since it is less potent in its action, it is more useful in determining the presence or absence of motor disturbances of the gall bladder and sphincter. Several recommend the administration of pituitrin one hour before the intravenous injection of tetraiodophenolphthalein in order to secure a preliminary emptying of the gall bladder and therefore a better visualization later (166) (435). The latent period before the pituitrin (subcutaneous) begins to cause evacuation is generally from fifteen to twenty minutes and may cause from one-third to complete emptying when it is effective. Atropine is said to antagonize the action of pituitrin in causing evacuation (303). (Adlersberg (5) (6) (8) has reported on the effect of certain hypnotics on the response of the gall bladder to pituitrin.)

In the dog, Kusnezow and Michailowa (316a) obtained some evacuation of dyed bile placed in the gall bladder (gall bladder fistula and duodenal fistula) after the intravenous injection of pituitrin in two out of four experiments.

Boyden (61) (539) and Bassin and Whitaker (35) found that an intravenous injection of pituitrin in cats caused iodized oil to be forced into the common duct, but the oil did not pass to an appreciable extent into the intestine. In this connection, it must be remembered that iodized oil is very viscous, and a potent excitant of the gall bladder is required to evacuate it, and also that a better response may be obtained by the subcutaneous administration of the drug.

Pilocarpine and physostigmine in therapeutic doses, according to the reports available (252) (295) (485), are not effective in causing evacuation in man. In dogs and cats (35) (536) these drugs may cause some iodized oil to be expelled into the cystic duct but evacuation does not result as a rule (220) (546) (551). According to Bassin and Whitaker (35) these drugs may even delay evacuation.

Atropin either has no effect or delays gall bladder emptying (35) (217) (295) (485) (536). Grebe (217) reports that the gall bladder fills better under the influence of atropin (1.0 to 1.5 mgm.), and after five hours enhanced evacuation to a stimulus is obtained, or even a spontaneous evacuation may occur.

Histamine. Lueth, Ivy and Orndoff (344) injected 1 mgm. of histamine acid phosphate subcutaneously into man and dogs, thinking that it might act directly on the gall bladder or indirectly by causing a flow of gastric juice into the duodenum. Out of ten men, emptying was obtained in only one; only two of ten dogs showed even a slight decrease in the cholecystogram.

Choline causes only a slight emptying of the gall bladder in man (252) and only some expulsion of iodized oil into the common duct in the cat (61).

Epinephrin subcutaneously (0.5 to 1 cc. of 1:1000 in man and up to 1 mgm. per kilo in the dog) has no effect, except that it may possibly delay emptying (132) (485) (536). Intravenously in the cat (0.5 cc. of 1:1000 or 5 cc. of 1:20,000), epinephrin causes a definite, but momentary evacuation (35) (61). *Ergotamine* (342), which may cause a slight contraction of the gall bladder *in situ* in the dog, according to Bassin and Whitaker (35) may produce slight evacuation in the cat. *Nicotine* (0.1 mgm. per kilo intravenously) causes slight evacuation of iodized oil in the cat (35).

Other chemical substances. Alcohol orally causes only a slight and variable evacuation of the gall bladder (485) (537). The effect of nitroglycerin is inconstant (35) (485), slight evacuation sometimes resulting. Calomel (0.16 mgm. per kilo) by stomach tube to cats causes no emptying of iodized oil (35). Magnesium sulphate intravenously depresses the gall bladder *in situ* in the dog. It may cause some filling of the ducts with iodized oil in cats, but no emptying, according to Bassin and Whitaker (35) who injected 15 cc. of a 25 per cent solution intravenously. Such a dose may cause some engorgement of the liver. Barium and calcium chloride and lead acetate intravenously may cause a slight evacuation (35) (61). Sodium salicylate

(0.6 cm. per kilo intravenously) (35) is without effect. Tumeric tea (curcuma temoelavac) (298) intra-duodenally, although it apparently stimulates bile flow, has very little effect on the size of the cholecystogram.

Cholecystokinin, a concentrated, vasodilatin-free extract of the upper intestinal mucosa injected intravenously, causes from partial to complete evacuation of the gall bladder in man and dogs (35) (264) (269).

The evacuating action of some of the drugs mentioned above may be due to an increase in liver volume. This possibility must be considered in the interpretation of the effect of various drugs or any substance injected intravenously. Another factor that must also be considered is the effect of the drug on the duodenum and the sphincter of Oddi. For example, theoretically, pilocarpin should cause more evacuation than it does, because of its action on the gall bladder musculature; but in all probability, it fails to do so because of the marked increase in tone of the duodenum and sphincter.

Reflex inhibition and excitation of the gall bladder from the gastro-intestinal tract. Doyon (154) observed a slow contraction of the gall bladder to occur after irritating the stomach with ammonia. Birch and Boyden (52) have made a serious attempt to determine the effect of electrical stimulation of the gastro-intestinal tract at different levels on the evacuation of the gall bladder. They worked with cats in which the gall bladder had been visualized with iodized oil and in which electrodes had been sewn into place. They believed that mechanical factors were ruled out because of the type of their results. On stimulation of the pars pylorica, they observed a sudden contraction of the gall bladder with ejection of oil into the cystic duct. If the stimulus were applied during the digestion of a meal of egg yolk, then the column of oil in the cystic duct passed back into the gall bladder. On this basis, these observers suggested that certain instances of spontaneous evacuation may be related to a gastric hunger contraction, particularly since in a human subject, a spontaneous contraction of the gall bladder was seen at the time when the human subject complained of a hunger pang. On stimulating the duodenum at the level of the ampulla of Vater, they obtained changes in the gall bladder contour during evacuation which were best interpreted as being due to relaxation. Similar inhibition was obtained on stimulation of various portions of the duodenum, jejunum and cecum, the ileum not being studied. Reflex contraction was not initiated from any portion of the intestine. In only one experiment was a contraction initiated from the cecum. The cecum was observed to be the most sensitive, not only in regard to the elicitation

of inhibition of gall bladder evacuation, but also in regard to pain. Subsequently, Boyden (72a) found that direct stimulation of the plexus that accompanies the left gastric artery induced relaxation of the contracted gall bladder.

These observations are obviously of particular clinical interest (76) because of the possible correlation of gall bladder disease with chronic constipation and appendicitis. However, Boyden (72), on applying the same technique to monkeys and human subjects (a preliminary report), finds that little, if any, effect on the gall bladder can be demonstrated by stimulating the stomach or duodenum. In this connection Levine (328) reports that, while he was studying the contraction wave of the visualized gall bladder in a patient, a sudden increase in the size of the gall bladder was noted, accompanied by the symptoms of nausea and vomiting.

Evacuation in striped gophers. Higgins (246) observed evacuation of the gall bladder in striped gophers after a fat meal. The gall bladder of this animal empties and refills in from five to nine hours.

Evacuation in the rabbit and guinea pig. Much difficulty has been encountered in attempts to demonstrate evacuation in the rabbit. Westphal (530) and Babkin (29) have reported that it occurs. Demel and Brummelkamp (147) and Halpert and Hanke (227) were unable to obtain evidence of evacuation. Walsh (523) was able to observe only a slight evacuation of iodized oil from the rabbit's gall bladder after injecting cholecystokinin. Thompson (505) observed only slight evacuation after feeding, although it is not stated whether he used brominated oil or some less viscous medium. Walsh (523), on injecting a series of doses of cholecystokinin, obtained evacuation of bile and methylene blue from the gall bladder, the gall bladder being reduced from one-third to one-half of its original size. Apparently complete evacuation of the gall bladder of the rabbit is difficult to obtain, or does not occur, its contraction being comparatively feeble. The gall bladder of the rabbit does not visualize with tetraiodophenolphthalein to a sufficient degree to render the method utilizable (121) (193) (343), which is very probably due to the fact that only about one-fortieth of the hepatic bile formed in the rabbit is concentrated in the gall bladder.

Evacuation in the guinea pig is readily demonstrated and has been discussed sufficiently.

Evacuation in the opossum. Evacuation in the opossum is comparable to that in the rabbit, except that complete evacuation may be obtained in the opossum by repeated electrical stimulation (162). When light brominated oil is placed in the gall bladder of the opossum, only from one-half to one-third is evacuated and then relatively slowly (161).

DuBois and Hunt (161) (162) think that the difference in rate of evacuation between the cat and opossum can be explained only partly by the difference in the thickness of the muscular tunic and in the tenuosity of the cystic duct.

Evacuation in the fish. Evacuation of the gall bladder occurs in fish (28) (245). Peristaltic activity is quite evident (245), but is slow to get started,—more than an hour. This may be due to a slow rate of digestion or the properties of the gall bladder musculature. Babkin (28) has observed evacuation of the gall bladder in fundulus, eelfont, skate, flounder and sculpin.

Evacuation in the monkey (*Macacus rhesus*). Because in the monkey the gall bladder is surrounded, sometimes to a considerable extent, by the liver, one might not expect rapid emptying. However, such has not been observed by Boyden (71) who has found that the initial rate of emptying in the monkey is comparable to that of man, all the iodized oil introduced into the gall bladder being emptied in two of three monkeys in as short a period as thirty-two minutes and forty-six minutes, respectively. Boyden (72) points out the supremacy of the "normal" primate gall bladder in regard to initial rate of evacuation.

Evacuation in cholecystitis. In animals, it has been demonstrated that in the presence of a marked cholecystitis, the gall bladder evacuates poorly, if at all. Potter and Mann (430) failed to observe wave-like rises in pressure in the common duct after feeding an animal in which cholecystitis had been produced. This indicated an absence of gall bladder contractility. Higgins and Mann (248) observed that dogs with cholecystitis do not evacuate readily after egg yolk. Whitaker (487) (538) has made a similar observation. Murphy (399) and Murphy and Higgins (401) found that the acutely inflamed gall bladder, induced chemically, does not evacuate. Complete recovery occurs, however, in about six weeks. These studies are not only of interest from a clinical viewpoint, but also from the physiologic viewpoint, in that they strongly indicate that the chief mechanism of evacuation is the contractile force of the gall bladder.

In man it is reported that in the presence of chronic cholecystitis with involvement of the muscular tunic, evacuation is slight (211, p. 106) (328). The presence of stones in the gall bladder may or may not decrease the rate of evacuation (328).

Types and phases of the evacuatory response of the gall bladder to a fat meal, egg yolk, and cream. Although a number of writers have referred to the hyperkinetic, vagotonic, sthenic, or hyperactive, and the hypokinetic,

sympathotonic, asthenic, or sluggish gall bladder, or to the various types of gall bladder response, the evidence presented is not yet conclusive The only serious attempt in the literature to study in detail, in man, the various phases and types of response of the gall bladder by a quantitative method is that of Boyden and his colleagues, which includes in his various publications an experience with over one hundred human subjects.

As has been found in the dog by McMaster and Elman (381) and in the cat by Whitaker (536), Boyden (69) observes an intermittency in gall bladder evacuation in man. He refers to the intermittent periods as *phases of contraction*. The first phase of contraction is divided into three parts: *a*. The *initial response* appears after a latent period of one minute after food and is completed in two minutes. It may be due either to a cephalic or psychic reflex relaxation of the sphincter and contraction of the gall bladder, or to the passage of food into the stomach or duodenum, since drinking water or injecting air intra-duodenally produces a similar response. *b*. The initial response is followed by a *two-minute pause* during which the gall bladder fills slightly or ceases discharging, due probably to a return of the sphincter to its original tone. *c*. Then follows the *principal period of discharge* during which from one-half to three-fourths of the contents are evacuated. This period lasts from sixteen to sixty minutes, or an average of thirty-two minutes (468). Following the first phase of contraction, there occurs a *period of quiescence* lasting from five to forty-five minutes. Then, the second phase of contraction begins, which may be followed by other phases until the gall bladder is practically emptied of the opaque dye. These later phases are not so sharply defined as the first, and the gall bladder being no longer distended, its muscle is less effective and evacuation occurs generally more slowly than during the first phase. Occasionally, a subject's gall bladder may empty half its contents during the first phase of evacuation and then refill, the shadow becoming less distinct. Then, during the second phase of contraction, the gall bladder may empty, the shadow disappearing completely. Boyden reports a subject in which the gall bladder emptied partially and filled twice during the digestion of a single egg yolk and cream meal. The secondary filling and incomplete evacuation are also mentioned in the literature on evacuation produced by pituitrin (435).

The length of the periods, or phases of more active contraction, are apparently longer in man than in the dog, the periods in the dog ranging from six to thirteen minutes (381). In the cat, the periods range from

ten to fifteen minutes in length, but may be very irregular, which Whitaker (536) thought might be due to the condition of the animal.

Boyden (69) describes *three types of response of the human gall bladder* to stimuli. In the *first type*, the introduction of a stimulus results in an initial relaxation of the gall bladder with an apparent simultaneous closure of the sphincter. Such subjects are relatively rare, and the condition results only in a temporary delay in emptying. The *second type* represents the other extreme. The reception of food causes contraction and very probably relaxation of the sphincter. Evacuation occurs at a rapid rate. The *third type* is the sluggish type in which the initial phase of emptying is followed by filling and the time of evacuation is prolonged. Boyden thinks that in this type, the sphincter is primarily concerned and not the gall bladder, because the speed of the initial response may be normal.

It should be stated that Boyden's method of computing volume from frontal plane cholecystograms has been criticized (368) (413). The details of the method have been published by Boyden (69). The method utilizes a mathematical procedure for computing volumes from a longitudinal silhouette, it being assumed that the gall bladder is "round" in transverse cross-section. This was a logical assumption for the contracting human gall bladder *in vivo*, but recent observations by Boyden on children, in whom it is possible to obtain distinct lateral cholecystograms, show that the anterior-posterior diameter of the evacuating gall bladder decreases at the same rate as the frontal, thus proving the assumption and establishing the relative merits of the computation.

Rate and completeness of evacuation. As pointed out above, Boyden has emphasized the species difference in the initial rate of evacuation. Most of the reports in the cholecystographic literature remark on the rather striking rapidity of evacuation that may occur occasionally in man. Chiray and Lomon (113) cite two cases in which the gall bladder shadow decreased one-half in size in a one minute period. Levine and Whitaker (327) (328) report that complete evacuation may occur in one-half hour. Boyden finds that from two-thirds to three-fourths evacuation generally occurs within the first thirty minutes after a meal of five egg yolks and one-half pint of cream (61) (69) (74), that the shadow is reduced 95 per cent in one hour and forty minutes and is gone in three hours. These are approximately the same data as reported by Case (106) and others (214) (328). Various observers state the normal time of evacuation of the gall bladder of man (disappearance of cholecystographic shadow) to be between three and six hours, following an

adequate fatty meal. Boyden (69) in a study of more than fifty normal human subjects gives a time ranging from sixteen minutes to four and one-half hours. Several observers point out, however, that the shadow occasionally may not disappear from a presumably "normal" gall bladder in twelve hours.

Other interesting points in regard to the rate and completeness of evacuation are as follows: Copher (121) has made the interesting observation that a persistent shadow may be maintained in the gall bladder by the daily injection of tetraiodophenolphthalein in the dog. Boyden and Birch (68) (69) failed to find a relation between rate of evacuation of the stomach and the rate of gall bladder evacuation in man; the same was true for gastric acidity. Boyden (69) did find a relation between initial size of the gall bladder and the rate of evacuation, the larger gall bladders evacuating more slowly. This relation does not always hold, however. Boyden (69) also reported an interesting study on a subject with an "hour-glass" gall bladder.

Just as the normal colon may not renew its entire content every day, the normal gall bladder may not renew its entire content daily. As the evacuation of the colon, or even the stomach, is related to the diet, the evacuation of the gall bladder is related to diet. Evacuation of the gall bladder may be and has been observed and conclusively demonstrated in every animal studied (fish, gopher, guinea pig, opossum, rabbit, cat, dog, monkey, man). There appears to the author no reason to doubt that the gall bladder of the species studied will evacuate or renew its contents daily, with the possible exception of the opossum and rabbit, on a diet containing adequate fat and meat protein. The evidence showing that the content of the gall bladder is evacuated normally via the cystic duct is incontrovertible, just as incontrovertible as the evidence showing that the stomach evacuates.

MECHANISMS CONCERNED IN GALL BLADDER EVACUATION. It has been demonstrated that the maximal pressure exerted by the contracting gall bladder upon its contents in the dog varies from 20 to 30 cm. of bile pressure. From this fact, the supposition that the musculature of the gall bladder in the dog plays an important rôle in the evacuation process appears to be obvious. But, before one can be certain that this supposition is a fact, or can be referred to as a logical deduction, the following conditions must be established. First, the mechanisms concerned in the excitation of the musculature of the gall bladder must be understood, at least in part. Second, it must be shown by direct evidence that the force of contraction is sufficient to force viscous bile from the gall bladder

when unaided by auxiliary forces. Third, it must be shown by direct evidence that the auxiliary forces acting alone do not evacuate the gall bladder in a "normal" manner. And, in order to obtain a complete physiologic picture of the process of gall bladder evacuation, one must consider and attempt to evaluate the rôle played by the auxiliary forces and mechanisms.

Mechanisms concerned in the excitation of the gall bladder musculature. The mechanisms which have been suggested for the excitation of the gall bladder are as follows: *a*, local stimulation by distention or by "pungent" bile; *b*, nervous reflex excitation from various portions of the gastro-intestinal tract; *c*, long nervous reflex excitation from the cord and higher centers, and *d*, humoral agents including the hormone, cholecystokinin, and circulating post-absorptive substances originating from ingested food.

Local stimulation. Galen (198) explained evacuation as being due to local stimulation, excited by "pungent" bile or by distention. The following facts suggest that a contraction from local excitation may result. *a*, like the intestine, ganglia are found in the gall bladder wall; *b*, distention may occasionally cause a contraction of the isolated viscus, or the viscus *in situ*; *c*, a certain but variable optimum tension is required for a maximum response to a stimulus; and *d*, spontaneous tonic contractions of the isolated viscus or the viscus *in situ* may occasionally occur. It has been pointed out above that strong irritants placed in the gall bladder may cause it to contract. It is unlikely that concentrated bile is sufficiently irritating to cause a similar degree of local irritation, but the physiologic concept of an "adequate stimulus" may be concerned. Another possibility that must be considered is that local stimulation may decrease the threshold of the neuro-muscular mechanism so that a previously inadequate reflex or humoral stimulus may become effective, thus accounting possibly for the spontaneous and intermittent types of activity referred to above.

Nervous reflex excitation from various portions of the gastro-intestinal tract. Long reflexes from higher brain centers. From preceding discussions, it should be recalled that the gall bladder contraction and evacuation is influenced to some extent by extrinsic nervous influences. Further discussion at this point is not considered necessary.

That an extrinsic nervous mechanism is essential for "normal" contraction and evacuation of the gall bladder is very unlikely. Whitaker (536) extrinsically denervated the gall bladder insofar as is possible in cats and dogs and obtained evacuation (125). Histologic control studies were made.

Humoral mechanism. The possibility that humoral agents may be concerned in exciting the gall bladder to contract and evacuate occurred to a number of investigators at about the same time. Secretin was suspected and tried by a number with negative results, although some obtained positive results (*vide supra*).

Boyden (61) recognized that the humoral agents might be of two kinds, namely, *a*, a specific hormone, and *b*, substances present in food or produced by digestion of food which act after being absorbed into the circulation. He performed blood transfusion experiments, obtaining suggestive but inconclusive results.

Are post-absorptive circulating substances originating from food concerned? In order to determine if fatty substances might be absorbed into the lymph and on reaching the gall bladder cause it to evacuate, Boyden (61) ligated the thoracic duct and the lacteals near the cisterna. On feeding egg yolk and cream, evacuation results. This experiment does not eliminate portal absorption, however. Whitaker (536) obtained evacuation on injecting intravenously an olive oil emulsion, but doubted the physiologic significance of his results because an emulsion of petrolatum had the same influence. Higgins and Wilhelmj (249), on injecting fine emulsions of various fats including egg yolk, failed to obtain evacuation. This has been confirmed essentially by Voegtlin, McEwen and Ivy (517), who by making graphic records of intra-gall bladder pressure, found that emulsified fats intravenously did not cause contraction. A rise in intra-gall bladder pressure only occurred when the liver volume underwent a marked increase due to pulmonary fat embolism, or as in the case of egg yolk, the animal manifested a marked fall in blood pressure. Fatty chyle obtained after the ingestion of egg yolk and administered in large amounts intravenously, does not cause the gall bladder to contract or evacuate (517). Nor were these observers able to extract a cholecystokinetic substance from olive oil, egg yolk or their digests. Small amounts of oleic acid emulsion intravenously were without effect. In man, Silverman and Dennis (478) found evacuation to occur after the oral administration of fatty substances in quantities insufficient to cause a significant change in blood fat. From the evidence at hand, it does not appear as if circulating post-absorptive substances having their origin directly from ingested food are concerned in promoting contraction and evacuation of the gall bladder.

Hormone mechanism. Brugsch and Horsters (85) concluded from their experimental results that secretin was a cholecystokinetic hormone, although they did not carry their studies beyond the pharmacodynamic

stage and did not control liver volume. Believing that the evidence at hand in 1926 indicated the existence of a hormone for gall bladder contraction, Ivy and Dysart (271) (488) transplanted the gall bladder into the omentum. Although they obtained some secretion from the transplant, contractions could not be recorded, probably because of the surrounding fibrous tissue. Later Ivy and Oldberg (264) obtained contraction and evacuation of the dog's gall bladder on the intravenous injection of vasodilatin-free extracts of upper intestinal mucosa. In the course of their experiments and those of others (342), adequate experiments planned to rule out adventitious factors were performed. This work has been confirmed (35) (256) (493).

By chemical methods, the active principle was concentrated so that 3 mgm. of solid substance on intravenous action caused from 1.0 to 11.5 cm. rise in intra-gall bladder pressure which persisted for varying periods of time. A series of injections in anesthetized or unanesthetized dogs caused rises in gall bladder pressure amounting to from 20 to 26 cm. of bile pressure, rarely 30. On further chemical study (265) (266) (269) (342), it was found that solutions rich in the gall bladder contracting principle and poor in secretin and vice versa could be prepared. This and other evidence (266) strongly indicated that the cholecystokinetic principle was different from secretin.

It was found that the introduction of dilute acid into the duodenum of the anesthetized dog would cause a rise in intra-gall bladder pressure quite regularly and generally more marked than digested fats. This provided an excitant that might be utilized for cross-circulation experiments.

Cross-circulation and viviperfusion experiments. Four carotid-to-carotid cross-circulation experiments were performed by Ivy and Oldberg (264) in which dogs with compatible blood were used. On introducing dilute hydrochloric acid into the duodenum of one dog, its gall bladder contracted. From six to ten minutes later the gall bladder of the other dog contracted. Graphic records were made. Positive results were obtained in three of the four experiments. This has been confirmed by the viviperfusion experiments of Houssay and Rubio (256). These investigators removed the gall bladder of a dog and anastomosed its arterial and venous supply to the carotid artery and external jugular vein of a second dog. On introducing dilute acid into the duodenum of the donor dog, the viviperfused gall bladder contracted in seven out of eleven experiments. The latent period of the response was from three to five minutes. The contraction of the viviperfused gall bladder lasted

from fifteen minutes to one hour, raising the intra-gall bladder pressure from 1 to 6 cm. Cholecystokinin injected into the circulation of the "donating" animal, caused the viviperfused gall bladder to contract. Egg yolk introduced into the duodenum of the "donating" animal failed to cause contraction in the viviperfused gall bladder. Ivy and Oldberg (264) found that egg yolk in the intestine failed to cause contraction of the gall bladder of the same dog, but slightly acidified (acid to litmus) digests did.

The author is of the opinion that, although dilute hydrochloric acid is the only excitant which has been demonstrated by cross-circulation experiments to cause a production of the hormone, acid is not the only excitant for hormone production. Just as there are other excitants for secretin production than acid, there are very probably other excitants for cholecystokinin production. This is not an entirely gratuitous view in the light of the denervation results of Whitaker (536). And, just as acid is the best excitant to use to demonstrate secretin production by the cross-circulation or the pancreas-intestinal transplant method, acid is the best excitant for the demonstration of cholecystokinin production on the basis of the facts observed by the author.

Acid was used primarily because it yields a rapid and quite regular response in anesthetized dogs, and because it is a pure chemical substance and intravenously in reasonable doses has no effect on the gall bladder. For these reasons one might obtain positive results in cross-circulation experiments, and results which could not be thought due to the absorption from the lumen of the bowel of an hypothetical cholecystokinetic agent, analogous in origin to secretagogues. Cross-circulation experiments were not undertaken with egg yolk and cream digests, but they should be. Such experiments will probably be attended by more failures than were obtained in the acid experiments. It is impossible to predict whether cross-circulation or the viviperfused gall bladder would be the method of choice. With either method, the technical difficulties are obvious.

Blood transfusion experiments in man. Using human subjects with visualized gall bladders, Sandblom (462a) has observed evacuation of the gall bladder to occur after transfusion of blood (450 cc.) from donors (three experiments) digesting egg yolks. Evacuation did not occur after transfusion of blood from fasting donors. This shows that a humoral mechanism for the initiation of gall bladder evacuation exists in man, and indicates that egg yolks very probably cause the formation of cholecystokinin.

Distribution, preparation and chemistry of cholecystokinin. The methods of preparing a cholecystokinin concentrate are given by Ivy, Kloster et al. (265). Although a preparation (269) has been made and injected into man, a practical and reliable method for producing a preparation suitable for routine use in man is not yet available.

The chief source utilized for preparing cholecystokinin has been the first six feet of the hog's intestine. Cholecystokinin has been found in the duodenum of the hog, dog, rabbit and man (155). It is not present in the colon (Lueth). Only traces have been found in the pyloric mucosa (155). Only traces have been found in the duodenum of the horse which has no gall bladder (156). The liver, kidney and spleen have been extracted without obtaining a cholecystokinetic principle. Like secretin, cholecystokinin production probably decreases at successive lower levels of the intestine. Indirect unpublished data indicate this to be true.

A dog unit, or threshold dose, of cholecystokinin has been tentatively and empirically defined as that amount of vasodilatin-free extract which will cause on intravenous injection a more or less immediate 1 cm. rise in intra-gall bladder pressure (269). For purposes of assay in terms of doses, or units, only dogs are used which give a standard response to a known "cholecystokinin powder" kept for such purposes. Such "control" powders must be checked from time to time on a series of dogs, since some of them decrease in potency after a month or two.

Very little is known concerning the chemistry of cholecystokinin, though the evidence indicates that it is closely related to secretin (265) (269).

Side effects of cholecystokinin. Kosaka and Lim (314) have reported that large doses of a cholecystokinin preparation caused an inhibition of gastric secretion and thought it might be related to the hormone, or rather chalone that is responsible for inhibiting the gastric secretion of transplanted gastric pouches when fat is introduced into the intestine. Burget (97) has reported that it increases duodenal motility in guinea pigs. Burgess and Ivy (95) found that cholecystokinin had no effect on the motility of the empty stomach during rest and hunger periods. Its effect on the motility of a Thiry-fistula of the jejunum was variable. Its effect on the tone of the sphincter of Oddi has not been determined. It causes intestinal strips to contract (228) (291).

Summarizing, the only possible interpretation of the evidence at hand, is that a hormone mechanism is concerned in gall bladder contraction and evacuation, the hormone being closely related to, but not identical with

secretin. The evidence would coincide better with other related physiologic observations, if it were shown unequivocally that digests of egg yolk and cream cause the hormone to be produced.

Is the force of contraction of the gall bladder sufficient to force bile into and through the cystic duct? Intrinsic ability of the gall bladder to evacuate. This question, apparently a simple one, has engendered considerable debate and regardless of the evidence about to be cited, doubt is still expressed in the recent literature (55) (368).

In the dog and cat the evidence demonstrates that the gall bladder may evacuate under the force of its own musculature. McMaster and Elman (381) cannulated the cystic duct, and then cut the common duct, inserting a cannula into the distal end and another into the proximal end. These cannulae were so connected by rubber tubing leading to the outside that the hepatic bile could be directed into the gall bladder or into the duodenum at will. Such a procedure isolated the gall bladder from the "flushing action" of the hepatic bile flow and from the "sucking or milking" acton of duodenal peristalsis. The gall bladder was permitted to fill during the interdigestive period, and when a meal was fed, the bile was forced out of the gall bladder against a pressure of 20 cm. of bile. Higgins and Mann (248) cannulated the common duct and tied all the hepatic ducts in dogs under local anesthesia, the abdomen remaining open. Then an egg yolk, cream and bile mixture was introduced into the duodenum. After about thirty minutes, the pressure in the common duct started rising and continued to rise, dark bile appearing in the cannula. After about three hours, a pressure of from 20 to 22.5 cm. of bile had been obtained. Ivy and Oldberg (264) cannulated the cystic duct and instead of feeding a meal, injected cholecystokinin at intervals. Dark bile appeared in the glass tube serving as a manometer and rose to pressures approximately the same as those stated above. Copher and Illingworth (123) cannulated the cystic duct, pointing the cannula toward the gall bladder. To the other end of the cannula, they attached a rubber balloon. They then filled the gall bladder with iodized oil. The next day roentgenograms showed the oil to be still in the gall bladder. They then introduced egg yolk and cream into the stomach by tube. Five hours later considerable oil had passed from the gall bladder into the rubber bag. Further evidence is the visualization of the hepatic ducts of the dog (264) and cat (35) (61) (474) in the presence of common duct obstruction or spasm of the sphincter of Oddi.

Intra-abdominal pressure. That the gall bladder is evacuated by changes in intra-abdominal pressure or movements of the abdomen inci-

dent to respiration, eating and vomiting is a time-honored idea, although there is no direct evidence to support it.

Some have performed experiments which led them to conclude that this idea is actually a physiologic fact; others have performed experiments showing this is not a factor; and still others immediately dismiss the idea as physically absurd.

In the experiments described under the preceding topic, it is difficult to conceive how respiration, or change in intra-abdominal pressure could explain the type of evacuation and rise in pressure observed. The change is too slow to be accounted for by an increase in intra-abdominal pressure due to a meal, or pressure exerted on the gall bladder by adjacent viscera. Also, experimental evidence shows that the ingestion of a meal, unless excessively large, does not influence intra-abdominal pressure, because of compensatory relaxation of the abdominal muscles (103) (304).

Winkelstein and Aschner (542) (547) have maintained that a rise in intra-gall bladder pressure incident to inspiration causes bile to be evacuated in the presence of a relaxed sphincter of Oddi. In fish (gar, pike and bullhead), the respiratory factor is entirely avoided; and in this species, Higgins and Mann (245) (248) have found that the gall bladder evacuates after a fat meal with the abdomen open.

It is definitely established and agreed that ordinary changes in intra-abdominal pressure do not cause evacuation of either iodized oil or iodized bile. But, during very deep respiration or vomiting, the intra-gall bladder pressure may rise as high as 10 or even 60 cm. of bile pressure. A physicist would say that evacuation could not occur even under this condition, because intra-duodenal pressure would be alike affected. The observed facts are as follows: It is reported by a number of observers (123) (233) (381) (485) (536) that vomiting and marked muscular activity do not cause evacuation. In the above cited experiment of Copher and Illingworth (123), in which the cystic duct was connected with an intra-abdominally located rubber bag, the passage of a stomach tube, the distention of the abdomen with air and its release, coughing and a tight abdominal binder did not cause the gall bladder to evacuate iodized oil into the rubber bag. Feeding a fat meal did. On the other hand, the author has seen slight evacuation of iodized oil to occur incident to vomiting in a dog with an indwelling cannula in the sphincter of Oddi, almost complete emptying occurring later after a fat meal. On applying deep pressure over the gall bladder region, some (209) (233) (544) have observed slight evacuation. Others have not

(34a) (233) (485). But, in such instances, one cannot be certain that the gall bladder did not contract, or that effective differences between intra-gall bladder pressure and intra-duodenal pressure did not occur which were not directly related to an elevation in intra-abdominal pressure. Sosman et al. (485) observed slight evacuation occur on provoking nausea and vomiting by irritating the duodenum with a duodenal tube in place. But with a duodenal tube in place, or with a duodenal or common duct fistula, the physical conditions are entirely changed, a fact that has been disregarded too frequently.

The physical fact that it is impossible to transfer fluid from one collapsible bag to another collapsible bag connected by a tube in a common pressure chamber by raising the pressure in the chamber, is difficult for some students to conceive. The objections raised are: adequate differences in collapsibility, variations in "postural tone," etc. The only way to convince such students is actually to perform the above experiment in which the gall bladder is substituted for one bag and a loop of duodenum or stomach for the other. Obviously if the duodenum or common duct is opened to the outside (or if during vomiting the pyloric and cardiac sphincters should be relaxed for a short time so that the duodenal lumen is subjected to atmospheric pressure!), a rise in intra-abdominal pressure may squeeze bile from the gall bladder, if the sphincter of Oddi is relaxed. However, in vomiting or on sudden changes in respiration, it is conceivable that pressure may be applied unequally because of visceral movements; and this may account for the slight amount of evacuation that has actually been observed.

The statement is warranted that changes in intra-abdominal pressure *per se* play no rôle in gall bladder evacuation. Under certain abnormal conditions, slight evacuation may occur concomitantly with sudden marked changes in intra-abdominal pressure, but the evacuation cannot be due directly to an uncomplicated rise in intra-abdominal pressure.

Flushing action of hepatic bile. Another possible mechanism that has been emphasized by Graham and his colleagues (211) (323) is that gall bladder evacuation is aided by the flow of hepatic bile into and out of the gall bladder. The direction of flow of the hepatic bile is determined *a*, by the variations in the tone of the sphincter of Oddi and duodenal motility; *b*, the secretory pressure of bile, and *c*, the variations in distensibility of the gall bladder. This process by dilution accomplishes a flushing or washing out of the gall bladder. That such a mechanism is not essential is shown by the experiments cited above. That this mechanism may be a factor in evacuation of the gall bladder is indicated by

the following experiment of Copher and Illingworth (123). They attempted to rule out the factor of ebb and flow of bile by tying all the hepatic ducts after the gall bladder was visualized by tetraiodophenolphthalein or iodized oil. When this was done and a fat meal fed, the gall bladder did not empty, or emptied only slightly (123) (211). Scott and Whitaker (474) repeated the experiment in cats and obtained 90 per cent evacuation. This experiment is similar to that of Higgins and Mann cited above, except that the latter workers cannulated the common duct. In such experiments, positive results obviously mean more than negative results. The only directly unchallenged experiment indicating that an "ebb and flow" of bile is capable of washing out the gall bladder is another interesting experiment by Copher, Kodama and Graham (126). They removed the gall bladder and substituted a rubber bag, connecting the bag to the cystic duct by a cannula. In another experiment (211), they encased the rubber bag in a cylinder composed of non-opaque material to avoid changes due to pressure of viscera, etc. They then injected tetraiodophenolphthalein intravenously and observed the density of the bag increase slightly up to sixteen hours. After that, the density decreased over a period of from six or eight days, the animals being fed. Whitaker (536), performing the same experiment with iodized oil, obtained no emptying of the artificial gall bladder. Ramond and Popovici (439) in similar experiments, using bile in the artificial gall bladder, obtained partial emptying on the intraduodenal instillation of magnesium sulphate and attributed the evacuation to the aspirating action of duodenal peristalsis. Evidently bile may be evacuated partially and comparatively slowly from an artificial gall bladder, but not a viscous substance like iodized oil.

In view of this evidence and that cited above on the action of bile salts and on the intermittency of gall bladder evacuation in some gall bladders, at least, it is apparent that the factor of dilution and interchange of bile between the common duct and gall bladder, as determined by the variations in tone of the sphincter of Oddi and duodenal motility, is concerned in the emptying of the gall bladder; but this process cannot be considered as an essential or as a prime factor. Considering the marked intermittency of evacuation of some human gall bladders observed by Boyden (69), it is likely that the washing out effect may operate to a greater extent in some human gall bladders than in others. In this connection, however, it should be recalled, to be exact, that hepatic bile which enters the gall bladder is normally quickly concentrated and its diluent action diminished somewhat. In evacuation after egg yolk

and cream, this mechanism is more the exception than the rule, and is not concerned to an appreciable extent during the first phase of contraction which expels from one-half to three-fourths of the gall bladder contents within a relatively brief period of time.

Factor of elastic recoil. Several writers (26) (100) (211) (311) who were unable to obtain evidence of much contractility in the gall bladder, but were convinced that the gall bladder evacuated, have attributed a portion of the evacuation to the "elastic recoil" (211) (311) of the viscus when the sphincter or duodenum was relaxed. An analogous expression is "overflow incontinence" (26). It is true that when the gall bladder is suddenly distended, it manifests a recoil just as any hollow viscus does. But such a phenomenon is as true of smooth muscle as it is of elastic tissue. In addition, it has been pointed out above that the gall bladder tends to adapt itself to different pressures which is a property of smooth muscle alone (postural tone). The fact that Boyden (61) has observed that a distended gall bladder tends to respond better to a fat meal is a fundamental phenomenon of muscle, namely, that the "energy of contraction is a function of the length of the muscle fibers." It is true that the elastic tissue may assist, but to what extent is undetermined. The suggestion that "elastic recoil" of the elastic tissue *per se* is a significant factor in evacuation is entirely hypothetical. Muscle tissue also manifests the property of elasticity, and no one has measured the elastic recoil of the elastic tissue of the gall bladder, whereas definite pressure responses may be obtained in many ways on applying adequate stimuli, which can only be accounted for by contraction of the smooth muscle of the gall bladder wall. In this connection it should be pointed out that smooth muscle may respond to a stretch stimulus when its "tonus excitability" is high. Applying this principle, the gall bladder may be distended until at some moment when its "tonus excitability" is high it responds to the stretch stimulus. Such excitability changes are not uncommon in smooth muscle and are thought to be the result of rest or metabolism and are known to occur independent of "reflexes."

Factor of change in liver volume. It has been pointed out above that an increase in liver volume in the dog by pressing on the gall bladder wall or by exerting tension on the serosa through ligamentous attachments may exert a pressure of some 8 cm. of bile pressure. This, along with other undetermined factors increasing liver volume (reflex or circulatory), may lead to some evacuation as is shown by the experiments of Whitaker (536), in which he injected olive oil intravenously. However, it has not been shown that during digestion there is enough increase in

liver volume to produce evacuation; and if true, it would hardly be expected to induce the specific changes observed on the ingestion of different foods. That a change in liver volume is a significant factor is entirely ruled out in the cholecystokinin experiments, the viviperfusion experiments, and in guinea pigs, cats and fish. Experiments on the isolated gall bladder of the dog show that its wall, when stimulated by smooth muscle contracting drugs, may exert a hydrostatic pressure of some 14 to 20 cm. of water (228) (285). So, of the total 20 to 30 cm. of bile pressure which the gall bladder *in situ* of the dog may exert, not more than approximately 8 cm. of pressure may be accounted for as being due to an increase in liver volume.

Factors of gravity and siphonage. Some have suggested that gravity and siphonage are concerned in the evacuation of the gall bladder. It is well known that when a fatty meal is given, the human gall bladder will evacuate if the subject is standing. In view of the mass of evidence at hand, the suggestion that gravity is concerned in emptying need not be seriously considered. In this connection, it is interesting that at one time it was thought that gravity was concerned in the emptying of the stomach (103).

It has also been suggested that the flow of hepatic bile past the orifice of the cystic duct exerts a suction (filter pump action) on the contents of the gall bladder. Actually *in vivo*, such an action does not occur as can be shown by placing methylene blue in the gall bladder and then perfusing bile through the hepatic and common duct at various pressures. Methylene blue stained bile is not so aspirated from the gall bladder (544). Several writers have pointed out that physical principles will not permit siphonage and suction to occur in the biliary passages, because the ducts are collapsible.

No one has actually demonstrated that it is possible to aspirate gall bladder bile rapidly by exerting suction through a cannula in the common bile duct. The author has failed in every attempt to do so. However, it is possible to evacuate the gall bladder completely in an anesthetized dog by inserting a cannula into the common duct and then exerting 2 or 3 cm. of water suction intermittently six or eight times per minute (268). From two and one-half to three hours are generally required to evacuate the gall bladder completely in this manner. In such an experiment, the gall bladder wall at first adjusts itself to the volume of the contents, but later when about empty, the exposed wall collapses or becomes concave. The appearance of the gall bladder during the second phase of evacuation by this procedure is very definitely unlike the ap-

pearance when the gall bladder is caused to evacuate by methods which cause the viscus to contract.

Milking action of duodenal peristalsis. It is conceivable that duodenal peristalsis by milking out the bile in the intramural portion of the common bile duct may exert a type of aspirating action on the contents of the biliary passages, as that just described. But in order for the gall bladder to be evacuated by such a process, hepatic bile would have to be formed at a slower rate than it can be milked out by duodenal motility, because the thin hepatic bile would be affected to a greater extent than the more viscous gall bladder bile.

This problem of the relation of duodenal motility or tone to the evacuation of the gall bladder is a very important one both from a physiologic and pathologic viewpoint. The solution of the problem involves primarily the answer to the following questions: *a.* Is duodenal motility or the common duct sphincter essential for gall bladder evacuation? *b.* Is the distal or intramural portion of the resistance to the flow of bile into the duodenum due solely to the motility and tone of the duodenal musculature? *c.* What is the true function of the "intramural mechanism" and how is it controlled?

In connection with these questions, it is of interest to point out that those investigators who have doubted that the gall bladder musculature is capable of causing evacuation have also doubted the existence of a special sphincter of Gage or Oddi, thus emphasizing above all others the factor of duodenal motility and tone. This is mentioned particularly because it is the basis for the two schools of opinion expressed in the literature since 1919, namely, those who believe that the gall bladder is actively and primarily concerned, and those who believe that it is only passively and secondarily concerned in the evacuation process.

Hence the question—*is duodenal motility or the common duct sphincter essential for evacuation?*—is an important one. In the paragraph above on the intrinsic ability of the gall bladder to evacuate, experiments were cited which, in the opinion of the author, unequivocally demonstrate that the gall bladder may evacuate when isolated from the milking action of duodenal motility. Additional evidence may be summarized briefly as follows: Boyden (68) filled the gall bladder (cat) with lipiodol and then sectioned the common duct. Some spontaneous emptying (this will be discussed later) of the lipoidol into the peritoneal cavity occurred, as it frequently does when the intramural resistance is ruled out. The next day a fat meal was fed and the gall bladder shadow elongated and iodized oil was expressed into the peritoneal cavity. Mann and

Higgins (365) cannulated the common duct of the guinea pig with the abdomen open and the gall bladder evacuated while visibly contracting with the common duct cannulated. In the fish, Higgins (245) has observed that after the introduction of an egg yolk into the duodenum, much peristaltic activity is manifested before the gall bladder starts (one hour later) to contract and evacuate. In the cat (536), a barium meal may pass through the gastro-intestinal tract without causing evacuation. In animal and man, carbohydrates do not evacuate the gall bladder as effectively as a fat or meat meal, yet they certainly cause duodenal peristalsis.

The fact that the gall bladder in acute experimental cholecystitis (401) or when the gall bladder wall is injured by clamps (536), does not evacuate, indicate strongly that duodenal peristalsis and the other possible accessory factors are not of prime importance in evacuation.

The data cited above on evacuation after gastrojejunostomy in man and dog and after introducing egg yolk into the jejunum show that evacuation may be obtained without food passing through the duodenum. (It was claimed that regurgitation did not occur.) It is possible that the introduction of fat into the jejunum may decrease the tone of the duodenum or sphincter of Oddi and facilitate the flow of bile; but if fat into the jejunum affects the peristaltic activity of the duodenum in the same manner as the stomach is affected, the peristaltic activity of the duodenum will be decreased. Thus, hypothetically, the favorable influence on the tone may be antagonized by the unfavorable effect on peristalsis. This possibility has not been investigated. Regardless of the outcome of such an investigation, it could not cast doubt on the more crucial evidence showing that *the gall bladder can evacuate through its own intrinsic mechanism*, and that *in the absence of the activity of the gall bladder musculature, the gall bladder is either not evacuated or is not evacuated at a normal rate* by the accessory factors that may assist.

Although the gall bladder may evacuate without the assistance of the duodenum or sphincter of Oddi, one cannot avoid the fact that there exists an important choledocho-duodenal mechanism for regulating the flow of bile into and out of the gall bladder. This introduces the second part of the question asked above.

Does a special sphincter of Oddi or Gage exist? Anatomy. The anatomic literature is not in agreement in regard to the existence of a special sphincter at the duodenal end or in the intramural portion of the common duct. Many authors (81) (197) (202) (239) (240) (277) (325) (345) (357) (364) (369) (402) (415) (416) (432) (455) (489) (553) describe a

definite anatomic arrangement of muscle fibers that might serve as a sphincter in the rabbit, cat, dog and other animals, some being more positive in their conclusions than others. A few (26) (224) (346) (492) take the position that there is no definite anatomic sphincter, unless bands of circular duodenal muscle which pass about the ducts may be regarded as the sphincter. Reconstructions of the musculature of the choledocho-duodenal junction have not been attempted.

In the guinea pig, it is agreed (101) (247) (248) (530) that a very evident ampulla is present, the ampulla being an integral part of the duodenal wall, with a sphincter at the proximal and distal orifice, which might be termed respectively the sphincter proprius choledochus and the sphincter papillae. In the opossum (160), the ampulla lies entirely outside the duodenal wall and its musculature is distinct from that of the duodenum. No sphincter action at the proximal end of the ampulla has been observed. This suggests (160) whether the anatomy of the ampulla in the opposum and the guinea pig might not represent the phylogenetic stages which precede the inclusion of the ampulla so completely in the wall of the duodenum in high forms that it is difficult to identify one muscular tunic from the other.

In the human, Giordano and Mann (202) (364) and Helly (239) have described bands of muscle fibers surrounding both the common duct and the pancreatic duct, suggesting a sphincter for each, but so intermingled as to render it unlikely that one might act without the other. Giordano and Mann (202) have also made the interesting observation that in some cases of cholescystitis and peptic ulcer in man, a definite hypertrophy of the musculature about the intramural portion of the common duct, extending extramurally, may be found.

Mann (354) studied the intramural portion of the common bile duct in more than twenty different species. He cautiously concluded that there is sufficient anatomic evidence to show a band of muscle fibers about the duodenal end of the common duct which could act as a sphincter. The arrangement of the muscle fibers varies considerably in individuals of the same species.

Physiological studies. The intramural resistance (the choledocho-duodenal resistance, the sphincteric resistance, etc.) to the flow of bile into the duodenum has been measured by many investigators under various conditions. The resistance may vary under different conditions from 5 to 80 cm. of water pressure. In the unanesthetized fasting dog, the intramural resistance may vary from 9.0 to 25.0 cm. of water pressure depending on the length of the fast. The same range of resistance is

observed in the majority of barbitalized dogs. In animals not possessing a gall bladder, the intramural resistance is lower (354) (356) (rat and pocket gopher, 3.0 cm. water).

Several attempts have been made to analyze the factors concerned in causing the intramural resistance in the dog. Higgins and Mann (366) dissected the intramural portion of the common duct so as to free it as far as possible from the muscularis of the duodenum. They then established a flow of fluid through the dissected duct and stimulated it electrically to determine if smooth muscle might be caused to contract and obstruct the flow of fluid. They found a restricted area in the duct which when stimulated caused obstruction. This area was located proximal to the entrance of the pancreatic duct, thus being analogous to the sphincter proprius choledochus referred to above. Lueth (341) by a special cannula, studied the intramural "pressure gradient" and found such a gradient to exist. The method and results permitted the conclusion that the intramural resistance could be divided into two portions, one at the level of the papillary orifice (sphincter papillae) and the other at a higher level in the duodenal musculature. The method did not permit the restriction of the resistance at the higher level to a definite area as in the experiments of Higgins and Mann. The resistance offered by the sphincteric mechanism of the papilla, which in some dogs with the duodenum slit open results in bulging of the mucosa proximal to the orifice, as the pressure is raised, amounts to from one-third to one-sixth of the total intramural resistance. On using drugs and sectioning and stimulating nerves and making simultaneous graphic records of intramural resistance and duodenal tone and motility, it was found that variations in intramural resistance can occur independently of detectable changes in duodenal tone. This confirmed Oddi and others who have claimed independence of control for the sphincter.

Westphal (530), working with the guinea pig and rabbit, concluded that a reciprocal activity exists between the upper or duodenal portion of the sphincter and lower or papillary portion of the sphincter. He reports that the vagus nerve causes contraction of the duodenal portion of the sphincter and relaxation of the papillary portion and that the splanchnic nerve has the opposite effect. This cannot be considered as proven.

The literature on the innervation and pharmacology of the sphincter or the choledocho-duodenal junction will not be reviewed. It is a difficult and involved question, because it is very difficult to separate duodenal changes from real sphincteric changes. In general, drugs and

nerve stimulation in the dog affect intramural resistance and duodenal tone in the same manner, although this is not always true. Atropin may occasionally decrease intramural resistance without an evident change in duodenal tone (341). Magnesium sulphate usually decreases intramural resistance, but does not uniformly do so. Dilute hydrochloric acid intra-duodenally is more variable in its action than magnesium sulphate (21) (87) (96) (99) (118) (125) (172) (276) (341) (354) (383) (436) (444) (530) (546).

Certain indirect evidence is best interpreted probably by assuming that a special intramural sphincter exists, which has a relation to the gall bladder. First, in those animals that have no gall bladder, the intramural resistance is lower than in those which possess a gall bladder (354) (356); the bile flow into the duodenum is continuous; sponging the duodenal orifice of the common duct does not inhibit the discharge of bile (356). Second, numerous observers have found the common bile duct dilated after cholecystectomy (14) (128) (130) (148) (169) (183) (218) (234) (236) (253) (287) (289) (290) (308) (405) (416) (454) (455) (465) (495) in the dog, cat, goat, rabbit and man. For the literature on the congenital absence of the gall bladder in man, see reference 465. In the guinea pig it is reported not to occur (101). It may also result after injury to the gall bladder by a drainage tube (359). Immediately following the removal of the gall bladder, the mechanism controlling the intermittent outflow of bile into the duodenum is destroyed (308) (436) (455). In some dogs, the incontinence disappears and the ducts dilate; in others, the dribbling persists (308) (455) and the ducts do not dilate, or not as much. If the sphincter is cut, the ducts do not dilate (290). Immediately after cholecystectomy, the common duct pressure rises, according to Potter and Mann (430), as might be expected, and the intramural resistance falls (290) (436) (546) (547). (They do not state how long their dogs had fasted, which is important in such studies (Elman and McMaster, 172).) Puestow (436) in Doctor Mann's laboratory, using a special type of biliary fistula preparation, has observed that immediately (twenty-four hours) after cholecystectomy, the orifice of the duct becomes patent, a continuous dribbling of bile occurs and the intraduct pressure falls very markedly. He believes that the patency of the duct could hardly be due to the paralysis or fatigue of the sphincter muscle in view of the rapidity of the change, but does not attempt to explain the difference between the intraduct pressure found in his and Potter and Mann's experiments. Since the only difference in procedure in the two experiments is that in Puestow's experiments the duodenum

was slit, the difference might be due to the section of the duodenal circular musculature. Either this must be true, or it is due to the fact that different dogs vary in regard to their sphincteric response. The important fact is the decrease in intramural resistance following cholecystectomy. And, unless it can be shown that cholecystectomy decreases duodenal tone, this fact strongly indicates the presence of a special sphincter. The fact that the ducts in some dogs and men dilate, also indicates the relative ineffectiveness of duodenal peristalsis in aspirating bile. Either this is true or there is some hidden or hitherto unsuspected cause of the dilatation of the ducts. Various attempts have been made to eliminate the sphincteric portion of the common duct either by transplanting the duct proximal to the sphincter into the duodenum or by placing an inlying catheter or cannula into the intramural portion of the duct. Following transplantation, it has been found that the intramural resistance is normal (99) and that the gall bladder will visualize and may be caused to evacuate by a meal (42). Such results have been ascribed to stenosis following the operative procedures, it being reported that the ducts dilate if the animals are kept a sufficient length of time (202). It has also been concluded that such results exclude (42) the possibility of a reciprocal reflex relation between the ampulla and the gall bladder; but this is not necessarily true, because in these experiments, the ampulla is still in place and the section of the common bile duct may not sever the reflex pathway. (We know from other experiments cited above that the reciprocal reflex mechanism is not necessary for evacuation of the gall bladder.) When the sphincter mechanism has been rendered ineffectual by section (536) or by placing a cannula in its lumen, more or less spontaneous emptying has been observed within six to eighteen hours. On feeding a fat meal, however, further evacuation was obtained. With the inlying cannula, the milking action of the duodenum on the duct is certainly obviated.

That duodenal motility and tone play a rôle in controlling the flow of bile into the duodenum is accepted as a fact by all observers. This is graphically represented by the simultaneous tracings of intramural resistance and duodenal motility published by Copher and Kodama (125) and Lueth (341). This fact confirms the more indirect observations of Elman and McMaster (172), Burget (99), Brugsch and Horsters (85) and others (312). But the visual observations of Giordano and Mann (202) and of Puestow (436) on the dog, of Higgins (245) on the fish, of DuBois and Hunt (160) (161) on the opossum, indicate that bile may be expelled sometimes without relation to discernible duodenal peristalsis or peristalsis may be present without the expulsion of bile.

Generalization. The relationship beween the sphincter of Oddi and duodenal peristalsis has been described as follows by Giordano and Mann (202) and also by Puestow (436); bile may spurt from the papillary orifice without evident peristalsis, but if peristaltic waves are present, the outflow will occur with the peristalsis. The author interprets the evidence as strongly indicating that in those species which possess a gall bladder, the common duct possesses a special sphincteric mechanism, which is intimately coördinated with duodenal tone and peristalsis, but which may also function independently.

Mechanism of "spontaneous evacuation." The mechanism of spontaneous evacuation may be considered properly in relation to the activity of the sphincter or the intramural mechanism, since it is very likely concerned to an important degree. (By spontaneous evacuation, the author means the evacuation of iodized oil or disappearance of the cholecystographic shadow without evident cause.)

It was pointed out above that in anesthetized dogs, a "spontaneous tonic contraction," amounting rarely to 6 cm. of bile pressure (total intra-gall bladder pressure, 11 to 16 cm.) and lasting five to ten minutes, may occur in the fasting anesthetized dog with abdomen open, and is of a type which shows that it is not an artifact. The contractions may occur without a change in blood pressure or in liver volume, or without borborygmi being heard. The most reasonable explanation is that they are reflexly induced from the stomach, since Birch and Boyden (52) have shown that by electrical stimulation of the empty stomach of animals, a partial evacuation may be obtained. The possibility of local origin within the wall of the gall bladder must also be considered. (See paragraph on "elastic recoil"). Still another possibility is the passage of the acid gastric residuum into the duodenum, which the author considers unlikely, but not ruled out by direct experiments.

In the case of "spontaneous contractions" or evacuation that occur in cholecystography, one must consider in addition to the possibilities just cited, the possibility of a reflex, excited distally or locally, resulting in a decrease in tone of the intramural mechanism which may lead to some evacuation provided the intra-gall bladder pressure is high. Boyden (69) is inclined to believe these changes are due to gall bladder contractions induced by or related to hunger contractions of the stomach. But in this connection, one must also keep in mind the fact that the duodenum is also active during gastric hunger periods. One is inclined to agree with Boyden's postulate that they are due to actual contraction of the gall bladder because of the type of change in the gall bladder con-

tour and because of Doyon's (154) work. Further evidence showing that manometric changes in gall bladder pressure may be induced by mechanical or electrical stimulation of the stomach or duodenum is desirable. On the other hand, it should be recalled that irritation of the stomach or duodenum is more likely to increase the resistance of the intramural mechanism than to relax it.

The question of the cause of the spontaneous disappearance of the cholecystographic shadow of the gall bladder during fasting has not been answered with certainty. A serious attempt to answer the question has not been made. During fasting, the shadow generally fades at twenty-four hours and disappears at about thirty-two to thirty-six hours. Graham (211) attributes the disappearance of the shadow to the dilution and interchange of bile as determined by changes in the duodenal sphincteric mechanism and the other factors cited above. Milliken and Whitaker (392), who followed the disappearance of the shadow in fasting in eight normal subjects, offer no explanation (see Crandall (132)). That it is due entirely to absorption of the dye by the gall bladder wall is not likely (*vide supra*), but it may be a factor. Duodenal hunger peristalsis may or may not be a factor, since an adequate study has not been made. Since drinking water may cause gall bladder changes in some human subjects, this factor must be controlled (69). The possibility that the dye may be slowly evacuated into the duodenum, reabsorbed and reconcentrated, thus leading to more prolonged visualization, must be considered. In this connection, it must be borne in mind that during fasting, bile secretion is diminished.

That the changes in duodenal intramural resistance must be considered in explaining spontaneous evacuation (69) is indicated by the following experiments in which iodized oil is placed in the gall bladder with an inlying glass cannula. If the gall bladder of a cat is only partially filled with iodized oil and the sphincter cannulated, none of it empties until a fat meal is fed; but if it is distended with the oil, a portion may be evacuated prior to feeding due to the "elastic recoil" and tonus of the gall bladder musculature. In similar experiments in dogs, one-fourth to one-third spontaneous emptying may be observed. Whitaker (537) observed spontaneous emptying of "thin" iodized oil in cats, but he offered no explanation other than that because putty-like masses were found in the gall bladder of the cats after several days, he thought the oil might have damaged the mucosa. Crandall (132) used brominized oil with a viscosity (5.3 at 38°C.) close to that of dog's gall bladder bile (iodized poppy seed oil, lipiodol, viscosity 90 at 20°C.) (gall bladder bile

viscosity, 1.4 to 2.2 at 20°C.). With no cannula in the sphincter in seven dogs, more than one-half of the light oil was spontaneously emptied in eighteen to twenty-four hours. With a cannula in place and using the more viscous lipiodol, about one-half emptying occurred. In both instances, a fat meal was followed by almost complete emptying (132). The author has been unable to find a record of the performance of similar experiments in which iodized bile was used instead of iodized or b ominized oil in dogs with a suitable type of duodenal fistula to avoid the possibility of reabsorption in the intestine. Such experiments would be more physiologic and assist in the solution of the problem.

The common duct sphincter or intramural mechanism may inhibit or prevent evacuation. It is obvious that since the gall bladder may contract with a force of not more than from 20 to 30 cm. of bile pressure, the intramural mechanism, which may exert a resistance of from 75 to 80 cm. of water, may prevent gall bladder evacuation. Thus, if the sphincter or duodenum is hypertonic, the gall bladder may contract, without evacuating, causing the hepatic ducts to become visualized as indicated above and may even cause biliary colic, or jaundice, if it persists.

Bollman, Sheard and Mann (57) have found that bilirubinemia may occur in the cholecystectomized dog when the intraduct pressure is around 20 to 25 cm. of water. In fasting, the intramural resistance in dogs markedly increases and may be as high as from 20 to 25.0 cm. of bile pressure (172). These facts may account (not the only explanation possible) for the starvation icterus, or the "physiologic jaundice" that occurs after twenty-four hours' fast in most dogs (382) and occasionally in man (438).

A large European clinical literature is developing on the dyskinesia of the biliary passages, in which motor disturbances of the choledocho-duodenal mechanism and gall bladder are thought to produce symptoms or to lead to pathology (23) (39) (82) (112) (293) (294) (301) (412) (467) (531). The experimental production of a biliary passage dyskinesia (spasm of the choledocho-duodenal mechanism with biliary colic) in a "normal" man has been reported recently by Ivy, Voegtlin and Greengard (267). Following an intravenous injection of secretin-cholecystokinin, pure pancreatic juice flowed copiously; bile flow had ceased and typical gall bladder distress resulted, the flow being resumed and the distress disappearing after intraduodenal instillation of magnesium sulphate. This is a condition that Schmieden (467) has termed "cholepathia spastica;" Newman (412) has termed it "spastic distention," and Westphal (531) "hypertonic dyskinesia." The possibility of such

phenomena occurring has been referred to also by Giordano and Mann (202) and by McWhorter (383). In this connection, Crain and Walsh (131) have demonstrated that duodenitis experimentally produced in dogs definitely delays gall bladder evacuation. Also, Shapiro and Kasabach (475a) produced a partial duodenal ileus in dogs and found that a temporary delay in the evacuation of the gall bladder invariably resulted. Later, in some dogs, the rate of evacuation returned to normal. In the other dogs, the emptying time remained prolonged which was interpreted as due to increased intramural pressure inhibiting evacuation.

The sphincter or intramural mechanism is necessary for filling of the gall bladder. It may be regarded as established that the sphincter of choledocho-duodenal mechanism is essential for the filling of the gall bladder. When an indwelling cannula is placed in the intramural portion of the common duct, the gall bladder does not fill (126) (147) (270) (548) (536) (539). In the dog if the intramural portion of the duct is slit open so as to destroy the sphincter of the papilla, the gall bladder fills and empties according to Whitaker (536). He attributed the filling to the resistance offered by the duodenal musculature, although according to Giordano and Mann (202) (366) a sphincter may be located higher in the duct. When the sphincter of the papilla was sectioned in the cat, the previously visualized gall bladder did not evacuate until after a fat meal (536), but the gall bladder did not fill again. Whitaker agrees with Mann (359) that the chief, if not the sole function of the intramural mechanism is to render it possible for the gall bladder to fill.

The time of, and the pressure relations necessary for filling of the gall bladder have not been investigated extensively. Volborth (520), using duodenal and gall bladder fistula dogs, found that during the first four or five hours post cibum, the bile flows only into the duodenum; then it flows into the duodenum and gall bladder, and later after the completion of digestion into the gall bladder only. In anesthetized dogs, Auster and Crohn (26) observed that the gall bladder begins to fill soon after it has been aspirated. McMaster and Elman (381) found in their "triple intubation" experiments that during digestion because of a decrease in the intramural resistance, bile tends to flow into the duodenum. The bile flowed into the gall bladder when the intramural resistance was about 6.0 or 7.0 cm. of bile pressure, the gall bladder not being in the state of contraction at the time of recording these pressures.

Another factor that is obviously concerned in the filling of the normal gall bladder is the tone of its musculature, or intra-gall bladder pressure.

An attempt to evaluate the various factors concerned in gall bladder evacuation. In the relatively rapid type of evacuation of the gall bladder of man and dog, which occurs after the ingestion of a meal of egg yolk and cream, or fat, the evidence shows that the chief factor concerned is a rise in intra-gall bladder pressure. This rise is due primarily to a more or less sustained tonic contraction of the musculature. The contraction is excited in part by a hormone (cholecystokinin) and by reflex nervous mechanisms (cephalic and gastro-intestinal). The sphincter of Oddi and the duodenal musculature promote evacuation by relaxing and permitting bile to be expelled by the contracting gall bladder. The evidence shows that a reciprocal functional relationship exists between the gall bladder and choledocho-duodenal mechanism; but whether this relationship is reciprocally causal is an open question. The evidence also shows that duodenal peristalses are not essential for, but assist evacuation. Peristalses may act by exerting a milking action on the intramural portion of the common duct. There probably exists some unknown intrinsic correlation between the sphincter of Oddi and the duodenal musculature, so that when the duodenum relaxes before an advancing wave of contraction, the sphincter relaxes. The evidence indicates that "elastic recoil," attributable to both the elastic and muscular tunic of the gall bladder, may in the presence of "high" intra-gall bladder pressure and a decrease in intramural resistance cause some evacuation. The dilution of bladder bile by hepatic bile, as determined by changing intramural resistance, the secretory pressure of the liver, and the phases of gall bladder contraction are concerned in the final or complete renewal of the contents of the gall bladder, or in the complete disappearance of the cholecystographic shadow.

The "collus-cysticus" sphincter. According to some anatomists, a sphincter is located at the neck of the gall bladder. Whether it is a sphincter or simply a thickening of the musculature is disputed (39) (240) (531). More recently, Lutkens (345) has described a sphincter at the junction of the collus with the valvular portion of the cystic duct, which occurs in 75 per cent of human subjects. His evidence has been questioned (69) (70) (415). Volborth (520) who made observations on the direction of the flow of bile in dogs with a gall bladder and duodenal fistula, thought that his observations might be explained in part by assuming the presence of a sphincter located somewhere between the common duct and gall bladder. Potter and Mann (359) (430), on making a manometric study of pressure relationships between the common duct and gall bladder, observed sometimes a pressure difference (10 cm. of

water) sufficient to suggest the existence of a sphincter at the neck of the gall bladder. Further experimental work on this question has not been found.

The valves of Heister. Throughout the greater portion of the length of the cystic duct in man, crescentic, valve-like projections of the inner wall of the duct are found, which give it a spiral and tortuous appearance. In some instances, smooth muscle extends (240) (497) into the leaflets, in others the muscle fibers do not extend beyond the base (223). According to the investigations of some (235) (504), the valves do not produce a spiral lumen, the lumen being free. However, it is very difficult to pass a small probe or catheter through without injuring the leaflets. The leaflets are much more evident in man than in any other animal studied. In rudimentary form they have been reported to occur in the dog and rabbit (240) (389). In man, they vary greatly in number (from four to twenty) (504) and in complexity. Occasionally, a leaflet may extend three-quarters of the way around the duct. Sweet (498) states that he has never found a human cystic duct free of valves. Mentzer (389) reports that no valves were present in eight out of 388 normal autopsy specimens and in six out of 123 cases of cholelithiasis.

On the basis of anatomic theory, various views of the function of the valves of Heister have been offered. Some have suggested that they impede inflow (355); some outflow (39) (238), others that they prevent evacuation (54) (147) (225) (497). Keith (305) has suggested that they keep the duct open by providing support, tending to prevent collapse. They have also been viewed as baffle plates to secure a slow passage of viscous bile from the bladder without opposing appreciably the flow of thin hepatic bile into the bladder (483a). Jacobsen and Gydesen (276) and others (126) have suggested that the S-shaped portion of the collus and cystic duct prevents over-distention. If this "kink" is straightened out, bile is easily expressed from the gall bladder. (See topic on evacuation of gall bladder in children.)

The pressure necessary to cause bile to flow in both directions through the cystic duct has been studied in animals and man. In the anesthetized dog, only 3.0 cm. of water pressure may cause the expression of bile from the gall bladder into the common duct (546), although at times, greater pressures (1.0 to 8.0 cm.) are required (279). In rabbits (147), it is reported that about two to four times more pressure is required to force fluid from the gall bladder than into the gall bladder. In man, such experiments have been confined solely to specimens obtained at autopsy. Lohner (339) observed that slightly greater pressure is re-

quired to cause fluid to flow from the gall bladder than into it. Mentzer (389), who used fluids of various viscosity at different pressures, obtained variable results, but concluded that the valves of Heister check the rapid passage of fluid into or out of the gall bladder. Johnston and Brown (279), employing an excellent method, could find no real impediment in either man or dog to the flow of fluid into or out of the gall bladder, when the pressures found normally in the biliary passages were used. At lower pressures (8.0 cm. of water or below), a lag in the equalization of pressure between the gall bladder and common duct was observed. This was attributed to the size of the ducts or to a "suction" action collapsing the ducts. Johnston and Brown (279) made the very interesting observation that in the human in contrast to the dog, the pressures between the duct and gall bladder equalized almost instantly, and tended to do so even at low pressures. This held true when the pressure was varied on either side of the cystic duct. If this observation were a confirmed fact, it appears to the author that the function of the valves of Heister is to prevent collapse of the cystic duct as suggested by Keith (305); and hence, the valves of Heister render the gall bladder of man a superior pressure equalizing apparatus particularly at low pressures.

THE RELATION OF THE GALL BLADDER TO THE FUNCTIONS OF OTHER ORGANS. *Gastric secretion.* It was not long after the introduction of gastric analysis that it was noted in general that patients suffering from biliary tract disease tend to manifest an anacidity or subnormal average acidity. In some patients, it has been reported that following cholecystectomy or medical treatment, normal acid values have returned which may be attributed to the improvement in physical fitness. The clinical reports at least show that following cholecystectomy in which the acidity was normal before operation, no significant change resulted (9) (10) (32) (46) (53) (57a) (58) (139) (200) (203) (212) (213) (242) (253) (286) (293) (324) (326) (335) (340) (352) (391) (417) (423) (448) (454) (455) (460) (480).

The difficulty in interpreting much of the clinical evidence in the light of present knowledge is that important factors, such as the age, general debility or physical fitness, appetite, and type of method employed for gastric analysis and stimulus used, were not properly considered. If a summarizing statement is at all warranted, it can only be said that there is a tendency in man toward anacidity in biliary tract disease, which is not specific in character and has no appreciable significance in the diagnosis and symptomatology of the affliction.

Considerable experimental work on this question has been done on

dogs where adequate controls are possible. Some have reported a decrease in gastric secretion following cholecystectomy (253) (455). Others have reported no change or a decrease (139). Still others have found either no change or a slight increase in gastric section (200) (391). These variable results may be interpreted to indicate that cholecystectomy has no specific effect on gastric secretion and that the variations are probably due to changes in the physical condition of the animals. That the presence of stones *per se* in the gall bladder does not influence gastric secretion in the dog is shown by the observations of Pepper (423) and Lichty (335), in which sterile pebbles were placed in the gall bladder where they remained for months without an evident change in gastric acidity. Badylkes (32) obtained variable changes in gastric secretion when foreign bodies were placed in the gall bladder or acute cholecystitis was produced in dogs. The evidence at hand certainly cannot be interpreted as indicating that the changes in gastric secretion associated with biliary tract disease are due to the breaking down of some direct hypothetical relation between the biliary tract and the gastric secretory mechanism which has been suggested.

It is clear from the clinical literature that disturbances of gastric motility are associated with biliary tract disease, particularly during the acute symptomatic manifestations (13) (46) (105) (212) (286). Indeed, some (13) (286) state that the principal symptoms of biliary tract disease are due to motor disturbances of the outlet of the stomach with frequent duodenal spasm and reversed peristalsis. It is reported that gastric lavage is as effective in relieving gall bladder colic as morphine (413). In experiments on anesthetized and unanesthetized dogs with a special balloon in place in the pyloric sphincter and antrum, Ivy and Fishback (272) have found that distention of the gall bladder or biliary passages will cause pylorospasm; also, that distention of the biliary passages will markedly delay the evacuation of a barium meal (balloon not in place, of course) (see Badylkes, 32). It is not known whether this is due to a local reflex or to a long reflex via splanchnics or vagi. It is well known that a similar pylorospasm, or disturbed motility is present in nausea and vomiting, and it would be necessary to section the splanchnics and vagi in order to determine whether the phenomenon is direct or indirect in nature. Because the source of the pain, nausea and vomiting induced by biliary tract distention may be from the stomach, Ivy and McEwen (273) resected the pyloric antrum in dogs and obtained the usual responses, but apparently not in such a marked degree. Typical gall bladder colic may also occur in man after resection of the pyloric antrum

(personal communication from Dr. Gatewood of Chicago and Dr. Balfour of the Mayo Clinic). Hence, it appears that the pain in gall bladder colic cannot be attributed to pylorospasm, which one would suspect on the basis of the different "reference areas."

The relation of gastric acidity to cholecystographic visualization of the gall bladder is discussed in the following references (143) (193) (250) (323) (396).

Cardiac activity. Space does not permit a review of the clinical and experimental literature in regard to the association between gall bladder and biliary tract disease and cardiac irregularities. The reader who is interested in this phase of applied gall bladder physiology is referred to the following references, which review the experimental and clinical literature (90) (133) (274) (412) (421) (473).

Pancreatic and bile secretion. Clinical evidence on the effect of cholecystectomy on pancreatic secretion is difficult to interpret because of the inadequate methods employed, the type of stimulus used, the bile dilution factor, the difficulty incident to making adequate control studies, and of the fact that varying degrees of chronic pancreatitis is so frequently associated with biliary tract disease. In cholecystitis, Piersol and Bockus (424) found a reduction in the enzyme concentration in duodenal contents in 85 per cent of forty cases. McClure and Huntsinger (372) have reported similar findings, and also that in cholecystectomized patients, the biliary pigment as well as the enzymatic concentrations are abnormally low (1). Sufficient studies before and after cholecystectomy were not made to determine the actual effect of cholecystectomy.

The only experimental studies available on the effect of cholecystectomy on pancreatic secretion and bile formation are those of Rost (455) who found a decrease in both. Further experimental work, in which careful attention is given to the condition of the animals, is needed in order to throw more light on this important academic and practical question.

That the gall bladder may have some reflex effect on bile formation is indicated by the observations of Demel and Brummelkamp (147). Using the rabbit, they introduced a catheter into the hepatic duct and followed the rate of flow of bile. On evacuating the gall bladder, the rate of flow was tripled. The increase in flow lasted about five minutes, returning gradually to the basal flow. Distention of the gall bladder inhibited the flow. This report should stimulate further investigation of the relation of the gall bladder to the rate of formation of bile. At

present. the data are too meager to warrant an interpretation or a suggestion of the physiologic meaning of such phenomena (see Man (359)).

THEORIES OF FUNCTION OF THE GALL BLADDER. It is worthy of note that among the digestive glands, the liver is unique in possessing a long, rather complex extra-glandular duct system, to which a receptacle, the gall bladder, is attached in most higher vertebrates. In this regard, the liver is imitated occasionally to a limited extent only by the pancreas, when the duct system of this gland manifests a structure known as a pancreatic bladder.

The function of the bile ducts is rather obvious. But the functions of their receptacle, the gall bladder, have been the cause of much speculation and experimentation.

In view of the facts at hand, it may be stated with a considerable degree of certainty that *the gall bladder serves as a "factor of safety" in digestion.* In addition, the author considers it definitely established that *the gall bladder also serves as a "factor of safety" in regulating the pressure in the biliary passages.* But it is not to be thought that these two functions are the only functions of the gall bladder. This is demonstrated by a consideration of the following briefly outlined discussion.

The storage of bile for purposes of digestion. The author believes that the evidence at hand is strongly presumptive in support of the classical concept of gall bladder function, namely, that the gall bladder stores much of the bile formed during the inter-digestive period to evacuate it during the early part of the following digestive period for the purpose of facilitating primarily the digestion and absorption of fatty foods. The propulsive force of the gall bladder serves to assist evacuation. Thus, during the inter-digestive period, the gall bladder husbands the bile for future use, and, because of its ability to concentrate, it may retain most of the output of the liver. The amount retained, however, varies widely in different animals. Thus, the gall bladder bile may be regarded as "ignition bile," using Pavlov's term. On being evacuated, it should not only serve the purpose of facilitating the digestion and absorption of fats, particularly, but should also increase the formation of bile. The facts that the ingestion of fatty foods evacuate the gall bladder more rapidly and completely than other foods, and that bile salts play an important rôle in the digestion and absorption of fats in the promotion of bile formation and in favoring intestinal motility, strongly support the concept. Also, it might be pointed out that emulsification in general is greatly favored by the concentration of the emulsifying agent and the same amount of emulsifier is much less efficient when diluted. Further,

it is best to add the emulsifier in small quantities at intervals. Another asset of a bile storage reservoir when the processes of the formation of the other digestive secretions are considered, is that the latter, the enzymes, are stored in the cells as precursors, which is not true for bile (483a). The storage function of the gall bladder is apparently designed to assist those digestive processes which are especially favored by a copious secretion of bile and a readily available supply of bile salts.

That the gall bladder can be removed without apparently influencing the digestion and absorption of fat is not an argument against the concept. There is no evidence at hand showing that cholecystectomy may delay the digestion and absorption of fat. On this point, the evidence available is inadequate upon which to base a positive or negative statement. But, just as "ignition (psychic) gastric juice" is not essential for adequate digestion, "ignition bile" may not be essential. As the psychic secretion of gastric juice is very probably one of the "factors of safety" in digestion, gall bladder bile may be viewed as a "factor of safety" in digestion, other factors being able to compensate to an adequate degree for its absence after cholecystectomy. Hence, it is believed that the gall bladder through its storage and concentration of hepatic bile is one of the mechanisms concerned in the correlation of the secretory activity of the liver with that of the other portions of the gastro-intestinal tract (359) during the various phases of digestive activity.

Regulation of pressure in the biliary passages. Undoubtedly, the gall bladder functions as a pressure regulatory mechanism for the biliary passages. In this regard, it functions as an elastic bag connected to a system of tubes, its elasticity being a property of its elastic and muscular tunics. Its effectiveness is increased by its ability to concentrate the hepatic bile rapidly and markedly. It is a pressure regulatory and storage reservoir of small volume but of large capacity (354) (456). It is able to provide for the varying rate of bile formation and for the exigency that the bile may not be able to escape into the intestine because of the resistance of the choledocho-duodenal mechanism. Thus, the secretory activity of the liver may continue unhampered by the inhibitory effects of high intraduct pressures for a period of hours. (Bile secreted against high pressure (20 to 25 cm. of bile pressure) is poor in pigment, cholates, and cholesterol (379) and pigment is retained in or returned to the blood (57).)

In animals that do not possess a gall bladder, the choledocho-duodenal resistance is small, whereas in animals possessing a gall bladder, the resistance is greater. In the former, the escape of bile into the intestine

is continuous, in the latter, intermittent. In view of this fact, it is not surprising that following cholecystectomy, the ducts dilate or the choledocho-duodenal mechanism relaxes in some manner. Both types of compensatory response occur.

Other facts which demonstrate the functional importance of the pressure regulatory function of the gall bladder, are as follows: *a.* Cholecystectomy hastens the onset of jaundice following common duct obstruction. *b.* The type of stasis fluid found in the bile ducts following obstruction varies with the presence (a green stasis system) and the absence (a white stasis system) of the gall bladder. *c.* The gall bladder does not by contraction normally exert a pressure on its contents greater than the secretory pressure of the liver. Such facts show that the gall bladder is an important "factor of safety" in the physiology of bile secretion and is essential for the maintenance of the normal structure and physiology of the biliary passages. It is believed that this principle deserves serious consideration before the gall bladder is to be viewed as a surgical trophy.

The foregoing outline indicates that the pressure regulatory function of the gall bladder is more important than its function of storage of bile for digestive purposes. Further knowledge of the fraction of hepatic bile stored by the gall bladder of different species and particularly a correlation of this study with eating habits, with types of food ingested, and with the motility of the biliary passages and changes following cholecystectomy is essential for a proper evaluation of these functions. It is known that the amount of bile secreted per kilo body weight varies widely (438). Mann's data (355) on the guinea pig and rabbit are a start in this direction and show that storage for digestive purposes cannot play much of a rôle in these two animals. Demel and Brummelkamp (147) report that in the rabbit, only from one-thirtieth to one-fortieth of the hepatic bile passes into the gall bladder; on the other hand, we know that the dog's gall bladder may retain at least the twelve hour fasting output of bile by the liver. In those animals which have a well developed muscular ampulla, such as the guinea pig and opossum, it is possible that the pressure regulatory function of the gall bladder is not so important. But, even in such animals, the gall bladder might provide a safety mechanism to care for temporary spasm of the ampulla or sphincter. This deserves further investigation. The secretory pressures of the liver of animals possessing and not possessing a gall bladder are too similar to explain the presence or absence of a gall bladder. The types of food ingested and eating habits of existing species do not assist because of

the wide variations, and we can only surmise the evolutionary trends in regard to the types of food and eating habits of the "progenitors" of the existing species.

As indicated above, the only established correlation is that between the presence or absence of the gall bladder and a high or low choledocho-duodenal resistance. Is high choledocho-duodenal resistance cause or effect, or did they develop mutually? As in the pigeon, does the gall bladder appear in embryo to disappear later in all forms which do not possess a gall bladder at maturity? (465) In the rat, according to Higgins (247a), it does not.

Because we cannot explain the absence or presence of the gall bladder in closely related species, because we cannot properly evaluate the two functions indicated above in all animals that possess gall bladders and because some animals and men live in good health for years after cholecystectomy, it does not follow, as some have stated, that the gall bladder is a vestigial remnant and should be removed prophylactically as is the appendix. It may be stated positively that the gall bladder is a factor of safety in physiological economy. It is not a vital organ, of course. Neither is the stomach a vital organ; but the stomach is an important factor of safety in digestion and like the gall bladder should not be removed except for absolute indications.

Is it sufficient to conclude that in all species in which considerable choledocho-duodenal resistance is found, some pressure regulatory mechanism is not only theoretically necessary but is also essential for normal structure and physiology? Is this the sole function of the gall bladder? The findings of McMaster (377) on the bilirubin concentration of rat's bile (eight times that of the mouse, which has a gall bladder) challenges an affirmative answer. His observations suggest that there may be something more behind the phenomenon of concentration of bile by the gall bladder than we now appreciate, and should stimulate further investigation, for example, in the pocket gopher or horse. This observation has other subtle interpretations. However, Higgins (247a) reports that in the rat an extensive intra-hepatic plexus of small biliary ducts, which surround the branches of the portal vein, is found. This is not true of the mouse. Hence, concentration of bile may occur in the intra-hepatic duct plexus in the rat. Also in the horse, which like the rat does not have a gall bladder, many sacculi, just visible to the unaided eye, project from the hepatic ducts (497). For these and other reasons, directly and indirectly implied by other observations recorded in various parts of this review, the author is convinced that search for

other functions than those enumerated above should continue. A general function may be found or facts already known may be supplemented and correlated to explain the presence and absence of the gall bladder in various animals.

The absorption theory. It has been suggested by some that the gall bladder absorbs and returns to the blood certain important constituents of bile. The evidence at hand does not indicate that a significant absorption of any of the ordinary bile constituents occurs. The absorption of water and inorganic salts can hardly be considered as specifically significant. Absorption of some unknown constituent of more or less specific significance may occur, but this has not been demonstrated.

Secretory theories. The function of the mucoid secretion of the gall bladder mucosa is very likely that of mucus in general, namely, for lubrication and protection. There is no evidence indicating that the secretion of the gall bladder serves any other purpose. There is no direct evidence which indicates the specific elimination of substances normally present in the blood stream. Neither is there evidence showing that the gall bladder elaborates a hormone which may be concerned with gastric secretion or intestinal motility (46) (260).

Other functions than those alluded to have been suggested. One of them at least deserves mention, namely, that the gall bladder regulates the formation of bile (147) (359) other than by evacuating its contents into the intestine. This possibility is related to the following questions: What is the meaning of the reported day and night rhythm of absorption in the gall bladder? What is the meaning and relation of the gall bladder to the reported cyclic (186) formation of bile by the liver which is said not to be due to alimentation?

The physiology of the gall bladder is far from being a completed subject. Numerous problems remain to be solved, the chief of which are: *a.* The discovery of a general function of the gall bladder, if there is one; and if not, an adequate explanation of the species differences. *b.* A more complete understanding of the physiology of the choledocho-duodenal junction and its control. *c.* The discovery of more factual knowledge concerning the motor disturbances of the biliary passages and their cause. *d.* The relation of disturbances of the motor, absorptive and secretory functions of the gall bladder to the cause of gall stones and a rational prophylaxis, a problem that is intimately related to the chemistry of bile and the physiology of bile secretion.

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Note: DuBois and Kistler (162a) report that stimulation of the ampulla itself, causes a contraction of the gall bladder which is abolished by section of the common bile duct. However, the gall bladder still responds in the usual way to stimulation of its wall, to excitation of either vagus nerve, and to stimulation of the hepatic end of the severed bile duct. These authors suggest that this points to the existence of a direct nervous pathway from ampulla to gall bladder along the wall of the common duct.

THE MOVEMENTS OF THE LARGE INTESTINE

R. C. GARRY

Institute of Physiology, The University, Glasgow, Scotland

In the latter half of the nineteenth and in the beginning of the twentieth century the problems of gastro-intestinal physiology in general, and of the physiology of the large bowel in particular, attracted many workers. This phase of experimentation on lower animals culminated in the work of Langley and Anderson, of Bayliss and Starling, of Elliott and Barclay-Smith, and, above all, of Cannon.

The apparent finality of the results obtained discouraged further investigation, and the crystallization of the findings in text-book dogma tended to mask the discrepancies and obscurities so obvious on examination of the original work.

In addition, the introduction of the opaque meal surrendered this field of investigation to clinical workers, so that practically all modern knowledge of the behaviour of the large bowel is due to work on man. Information gained from investigation of clinical material is difficult to evaluate, is often scanty, and is, at all times, at the mercy of the capriciousness of disease processes and of injury.

In very recent times the realisation of the possibilities of visceral nerve section in the treatment of various gastro-intestinal abnormalities has turned the attention of surgeons to experimental work on the alimentary tract. If this experimentation is not to be on the human subject, there is clamant need for a review of our knowledge of the physiology of the large bowel, and for some attempt to assess the validity of the sources from which our present conceptions derive.

1. MORPHOLOGICAL CONSIDERATIONS. Unless due allowance be made for differences in morphology, it is unsafe to apply to man results obtained in lower animals.

a. *The form of the large bowel.* In lower forms of life the terminal region of the intestinal tract is usually short and straight—a true rectum. In mammals the colon is interposed between the small intestine and the rectum proper, and, in addition, from reptilia onwards, a blind gut or cecum is usually present at the cranial end of the colon.

Even among mammals the large bowel varies greatly in structure. The best general description is that of Flower (1872). Descriptions of the large bowel in the more familiar domestic animals are given by Ellenberger (1892). Mivart (1881) and Reighard and Jennings (1925) deal with the cat and Ellenberger and Baum (1891) with the dog.

In spite of minor differences in individual species it is possible to recognise the form of large bowel typical of carnivorous animals and the form

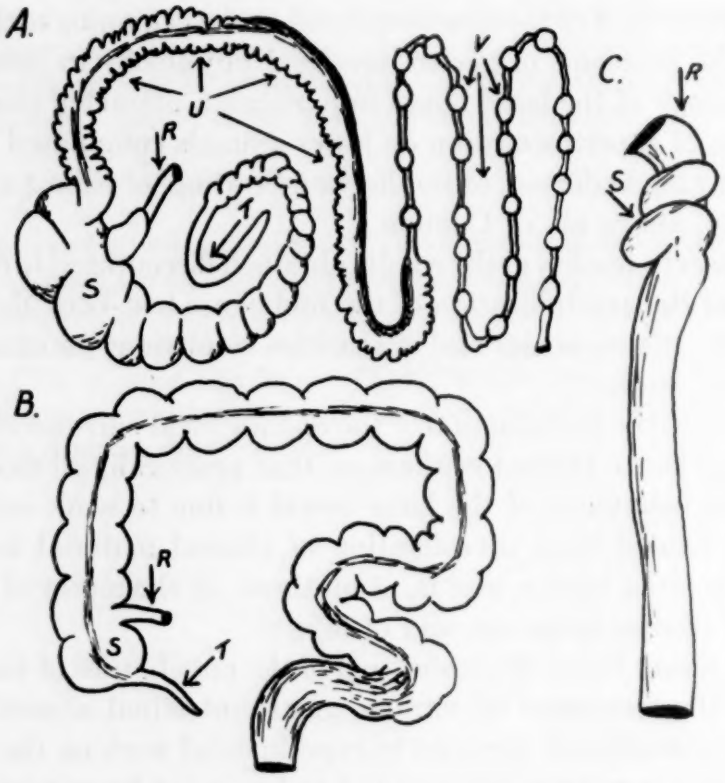

Fig. 1. The large bowel. A. In the rabbit. *R.*, ileum; *S.*, cecum; *T.*, appendix; *U.*, proximal colon; *V.*, distal colon. B. In man. *R.*, ilum; *S.*, cecum; *T.*, appendix. C. In the dog. *R.*, ileum; *S.*, cecum.

typical of herbivorous animals (Macewen, 1904; Elliott and Barclay-Smith, 1904). The terms of the human anatomist should be avoided. It is better to refer to the proximal, intermediate and distal regions of the colon since such a division has a functional significance.

In *carnivorous animals* the large bowel is relatively short and the outer longitudinal coat is a uniform powerful muscle layer. The cecum is vestigial, the ileo-colic junction is a straight tube, and macroscopically there may be little to distinguish ileum from colon. In the cat, however,

the colon approximates in form and in relative size to that of man (Starling, 1910; Lenz, 1924).

In *herbivorous animals* the cranial part of the large bowel shows a complexity of structure apparently associated with the digestion of cellulose, for in ruminants this elaboration of cecum and proximal colon is less marked. In general the ileum opens into a large sacculated cecum often partially separated from the proximal colon by a fold of mucous membrane. The proximal colon itself is large and sacculated, and the longitudinal muscle coat is partially absent. The intermediate and distal colon is much narrower, has a uniform muscle coat and is devoid of sacculation, but there may be subsidiary loops. In the rabbit, the proximal colon, where the fecal matter is soft and bulky, is separated from the distal colon, where there are hard dry scybala, by a fusiform thickening of the muscular coats—the fusus coli (Auer, 1925).

In *omnivorous animals* the colon lies structurally between the extremes of the carnivorous and herbivorous types. The colon in man approximates in form to the herbivorous rather than to the carnivorous type; the cecum is not large but the ascending, transverse, and descending limbs of the colon show sacculation, and the longitudinal muscle first forms a uniform coat in the caudal part of the pelvic colon and in the rectum. The iliac and pelvic colon may be regarded as a subsidiary loop characteristic of the distal colon in herbivores.

The pelvi-rectal flexure or sphincter, which plays an important part in the function of the human colon (O'Beirne, 1833; Hurst, 1919), is not present in laboratory experimental animals. In the dog, however, there is a constriction 5 cm. craniad to the anus. Caudad to this constriction the colonic walls are lax, craniad they possess high tone (Matti, 1909).

It is now generally accepted that the ileo-colic junction is guarded by a true sphincter muscle.

The anal canal is encircled by smooth muscle fibres which form an ill-defined band close to the anus. This visceral muscle sphincter is a thickening of the circular coat of the colon and it is advisable to confine the name "internal anal sphincter" to it alone.

b. *The myology of the pelvic region.* The levator ani of man is essentially a modification of the pubio- and ilio-caudalis muscles of lower tailed mammals in which these muscles flex and abduct the tail, the only possible action on the rectum being lateral compression. In man, however, the fibres arising from the pubis have lost connection with the rudimentary caudal vertebrae and pass round the rectum to form a supporting muscular sling (Thompson, 1899; 1898–99).

In tailed mammals a powerful band of smooth muscle, the recto-coccygeus (caudo-rectalis), unites the caudal region of the large bowel and the tail vertebrae. It is a continuation of the longitudinal coat of the gut and vestiges are present in man. Another smooth muscle in this region is the retractor recti et penis. The retractor recti alone is represented in man.

In all mammals the anal canal is surrounded by striped muscle fibres which overlap to a greater or lesser degree the visceral anal sphincter. This striped muscle may be divided into three layers. Unfortunately the cranial portion is sometimes called the internal anal sphincter.

c. *The innervation of the large bowel: The intrinsic nerves.* Auerbach's plexus is better developed caudad than craniad in the large bowel (Gerlach, 1873; Roith, 1909; Ten Caté, 1922). The more powerful the muscle coats, the closer is the mesh of the myenteric plexus and the more numerous are the ganglion cells. Meissner's plexus is also present in the colon. Gaskell's statement (1916) that Auerbach's plexus in the distal part of the large bowel has taken up a position external to the gut itself and is represented by the pelvic plexus is not tenable. Keith (1915a; 1915b) describes a tissue, neither true nerve nor true muscle, which forms a collar round the ileo-colic junction and is especially abundant in the distal colon where tone is normally high. Keith regards this as the most excitable tissue in the colon, a nodal tissue, overactivity of which may lead to a definite type of colonic stasis.

The extrinsic nerves. Two morphologically distinct sets of nerves supply the large bowel in lower animals. The cranial set, the "sympathetic outflow," arises from the thoracico-lumbar nerve roots; the caudal set, the "parasympathetic" outflow, arises from sacral nerve roots. Present uncertainty about the function of these outflows makes the use of the terms "sympathetic" and "parasympathetic" unwise. It is more satisfactory to use the noncommittal words "upper" and "lower," as did Langley and Anderson, or, better still, to confine oneself to the purely morphological terms "thoracico-lumbar" and "sacral."

The classical description of the innervation of the pelvic organs in experimental animals is that of Langley and Anderson (1894a; 1894b; 1895a; 1895b; 1896). Marked variation may occur even in the same species of animal. The diagram in figure 2 A and the following description, both derived from the work of Langley and Anderson, apply to the cat.

The fibers of the *lumbar outflow* arise from lumbar nerve roots, enter the sympathetic chains and then leave the chains to run in the meso-

colon to the inferior mesenteric ganglia. Langley and Anderson call these fine strands of nerve fibres the "spinal rami of the inferior mesen-

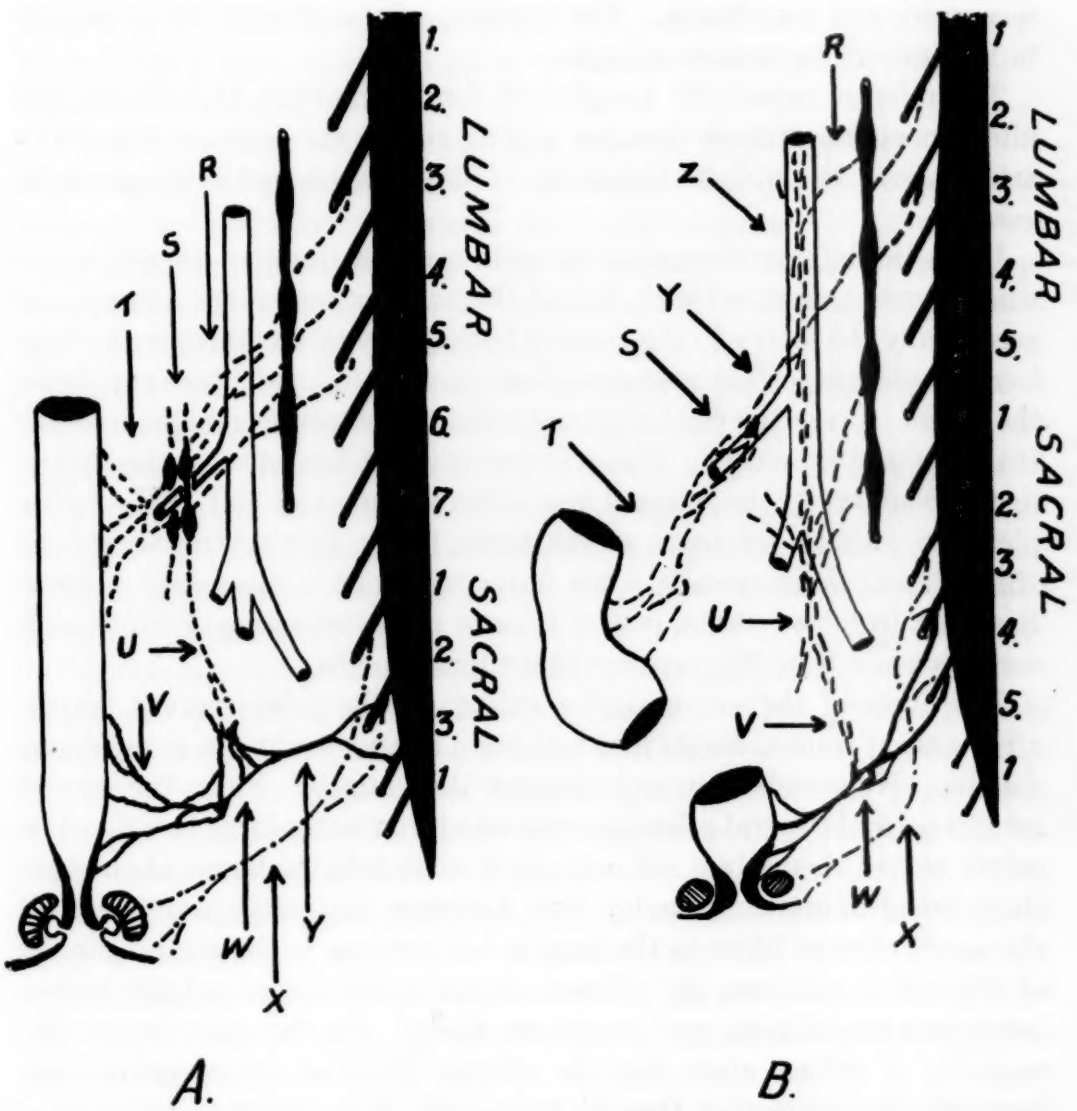

Fig. 2. The nervous outflows to the distal colon. A. In the cat. *R.*, spinal rami to the inferior mesenteric ganglia; *S.*, inferior mesenteric ganglia; *T.*, lumbar colonic nerves; *U.*, hypogastric nerve; *V.*, sacral colonic nerves; *W.*, pelvic plexus; *X.*, pudendal nerve; *Y.*, pelvic nerve. B. In man. *R.*, pelvic splanchnic nerves; *S.*, inferior mesenteric plexus; *T.*, inferior mesenteric nerves; *U.*, presacral nerve; *V.*, hypogastric nerve; *W.*, hypogastric plexus; *X.*, pudendal nerve; *Y.*, intermesenteric nerve; *Z.*, intermesenteric plexus.

teric ganglia." They regard "inferior splanchnic nerves" as a suitable alternative. Gaskell (1916), on the other hand, prefers to call these

branches the "lumbar splanchnic nerves." The spinal rami are composed mainly of fine efferent medullated fibres with a variable number of non-medullated fibres. There are probably also afferent fibres from the mesentery and the viscera. The majority of the efferent fibres synapse in the inferior mesenteric ganglia.

The inferior mesenteric ganglia are four in number, the ganglia and interconnecting strands forming a ring round the inferior mesenteric artery about 1½ cm. from the aorta. Often there is partial fusion of the ganglia.

From the inferior mesenteric ganglia arise the lumbar colonic nerves which form a close network round the inferior mesenteric artery and accompany the artery to the gut. The majority of the fibres in the lumbar colonic nerves are post-ganglionic non-medullated fibres supplying the colon. From the caudal pair of inferior mesenteric ganglia arise the two hypogastric nerves. These nerves give off branches to the ureter, to the bladder, to the internal generative organs and end in the pelvic plexuses. About one-tenth of the fibres in the hypogastric nerves are afferent, and there are also a few fibres belonging to the sacral outflow. Branches from the cranial pair of inferior mesenteric ganglia unite with nerve strands from the superior mesenteric ganglia.

The fibres of the *sacral outflow* emerge in the pelvic nerves, which, after a short course, divide into two main strands ending in conspicuous ganglia. Numerous filaments connect the ganglia. From the caudal ganglia arise the sacral colonic nerves which run to the large bowel. The pelvic nerves themselves are composed of medullated fibres, about one-third being afferent. Langley and Anderson find that practically all the sacral efferent fibres to the large bowel synapse in the caudal ganglia of the pelvic plexuses, the efferent fibres in the sacral colonic nerves being non-medullated post-ganglionic fibres. On the other hand, the majority of writers state that the efferent fibres of the sacral outflow pass without interruption through the ganglia of the pelvic plexuses along the sacral colonic nerves to synapse with the cells of Auerbach's plexus in the colon.

The *pudendal nerves* are somatic nerves innervating the external anal sphincter and other perineal striped muscles. They supply sensory fibres to the perineum. Branches from the pelvic plexuses join the pudendal nerves (Nikolski, 1879; Langley and Anderson, 1896).

The classical description of the outflows to the large bowel has recently been called in question by Ken Kuré and his fellow workers (1928, 1930, 1931). They claim that there are efferent medullated fibres in the

dorsal roots of the spinal cord. Such fibres synapse in the dorsal root ganglia but do not lose their myelin sheath. The fibres for the gut pass without interruption through the ganglia of the lateral sympathetic chain. Functionally these fibres resemble the bulbo-sacral division of the autonomic system and have been given the name "spinal parasympathetic" fibres by Ken Kuré.

Macroscopically, both in man and in lower animals, fibres from the vagus reach the proximal region of the colon. Section of the vagi leads to degeneration of nerve fibres in the proximal and intermediate colon but not in the distal colon. Section of the pelvic nerves causes degeneration of all fibres in the distal colon and of many fibres in the proximal and intermediate colon (Schmidt, 1933).

The outflows to the colon in man. Conventional anatomical description of the visceral outflows in man does not enable one to correlate the human arrangement with the form and function of the outflows in lower animals. Fortunately two surgeons, Rankin and Learmonth (1930; 1932), and Learmonth (1931) have made such correlation possible so far as the distal colon is concerned. Figure 2B and the verbal description are taken from their papers.

On the antero-lateral aspects of the abdominal aorta lies the *intermesenteric plexus* (abdominal aortic plexus). This plexus receives branches from the lumbar sympathetic chains by way of the *pelvic splanchnic nerves.* Close to the inferior mesenteric artery a large trunk, on either side, leaves the intermesenteric plexus to accompany the inferior mesenteric artery. These two trunks, the *intermesenteric nerves,* unite to form the *inferior mesenteric plexus.* In man this plexus contains two to four ganglia as in lower animals (Margorin, 1931). The pelvic splanchnic nerves and the intermesenteric nerves contain medullated fibres and correspond to the spinal rami to the inferior mesenteric ganglia in lower forms.

From the inferior mesenteric plexus arise the *inferior mesenteric nerves* which run in the mesentery to supply the distal colon. The inferior mesenteric nerves correspond to the post-ganglionic lumbar colonic nerves of the cat and of the dog.

The intermesenteric plexus continues caudad as the middle root of the *presacral nerve.* The lateral roots of the presacral nerve come from the lumbar sympathetic chains. The presacral nerve, more often a plexus than a nerve, descends into the pelvis and divides into the two *hypogastric nerves* which run caudad to join the corresponding *hypogastric plexuses* at the side of the rectum and bladder. The presacral nerve

and the hypogastric nerves represent the hypogastric nerves in lower animals. The hypogastric plexuses correspond to the pelvic plexuses.

The main sacral outflow in man is from the third sacral nerves and joins the hypogastric plexuses (Harman, 1898–1899).

2. Experimental methods. In assessing the value of results obtained by experimentation on the gastro-intestinal tract it is very necessary to take into account the methods used.

a. *The preparation of the animal.* It is inadvisable to administer purgatives shortly before experimenting on the gut (Alvarez and Taylor, 1917–1918). Much of the older work is suspect for this reason. Whatever the cause, an empty gut is an inactive gut and relatively unresponsive to any form of stimulation.

All anesthetics interfere with the normal behaviour of visceral organs and depress the spinal reflexes controlling the activity of such structures. Non-volatile anesthetics, which have come into favour recently, are as objectionable as volatile anesthetics.

To avoid the use of anesthesia, decapitate or decerebrate preparations may be used. The need for artificial ventilation in the decapitate preparation is a drawback, and even decerebrate preparations are not free from objection because recent work has shown that nerve tracts, connected with control of the viscera, arise in the brain stem (Barrington, 1921, 1928; Dresel, 1923; Beattie, Brow and Long, 1930).

Opening the peritoneum leads to reflex inhibition of the gut. Such inhibition must have played a part in the results of earlier workers. In addition, mere exposure of the gut, apart from nervous influences, may depress gut movements due to loss of CO_2 from the peritoneum (Meltzer and Auer, 1907b; Henderson, 1909).

b. *Methods of recording.* There are few methods of recording peculiar to the large bowel. The various methods applicable to the gastro-intestinal tract in general may be used. A complete description of all methods is to be found in Abderhalden's "Handbuch der biologischen Arbeitsmethoden"—Uhlmann (1926) deals with the methods in more common use, Katsch (1926) describes the use of the abdominal celluloid window and Kochmann (1929) deals with in vitro technique.

Earlier workers, such as Langley and Anderson, relied on naked eye observation with all its attendant disadvantages. Legros and Onimus (1869) passed recording balloons into the gut through permanent fistulae —a method which finds favour at the present day. Van Braam-Houckgeest (1872) placed the entire experimental animal, with the exception of the head, in a bath of warm saline; the fluid protected the intestines

after the abdomen was opened. This method also has stood the test of time. In 1898 Cannon introduced the use of opaque meals and of enemata to study bowel movements. This is perhaps the most satisfactory method of all, although it is more suitable for clinical than for experimental work. Very recently Barcroft and Steggerda (1932) have succeeded in "exteriorising" the proximal part of the large bowel in dogs.

Magnus (1904) first fully exploited the in vitro method and Trendelenburg greatly enhanced its value by recording the response of the gut to distention.

The sharp transition from gut to sphincter makes difficult the recording of the behaviour of the ileo-colic and anal sphincters. A "pencil" recorder, such as Gowers (1877) used for the anal sphincters and Elliott (1904) for the ileo-colic sphincter, is most satisfactory.

c. *Methods of stimulation.* By common consent, distention is regarded as the normal mechanical stimulus for both small and large intestine. Thus the balloons, used by so many workers to record the movements of the gut, usually served also to stimulate the gut. Certain workers use pledgets of cotton wool covered with soft soap or a paste made of starch or of pease meal.

Methods of investigating the action of nervous outflows to the gut leave much to be desired. Nerve section is infinitely to be preferred to nerve stimulation. Simple section interrupts impulses normal in quantity and in quality, while stimulation initiates nerve impulses probably abnormal both in quantity and in quality. In addition, there is, at the present time, no satisfactory method of gauging the actual quantity of the stimulation applied to a nerve. As long ago as 1897, Bunch showed that the nature of the stimulus affects the response from the gut. Recent work confirms this (Finkleman, 1930; McSwiney and Robson, 1931). One cannot doubt that much of the older work on nerve stimulation is open to grave suspicion and is in urgent need of repetition.

3. THE INTRINSIC PROPERTIES OF THE LARGE BOWEL. a. *The colon.* The distal colon of the rabbit shows active pendulum movements in vitro. A pea or fecal pellet inserted into the cranial end of an excised segment is driven out at the caudal end by typical peristaltic waves. This power of propelling gut contents caudad disappears long before the pendulum movements cease (Langley and Magnus, 1905). The distal colon of guinea pigs shows similar properties (Tyrode, 1910).

Elliott and Barclay-Smith (1904) and Cannon (1912a) noted that an excised and distended segment of colon from the cat may show antiperistaltic waves. Lurje (1926a, 1926b), using Trendelenburg's ap-

paratus, failed to detect such waves, but did elicit pulsating contraction rings. There is apparently no critical distending pressure, as there is in the small intestine, for eliciting movements.

Strips of muscle from the colon will show rhythmical activity in vitro, but such activity is not so easily obtained as it is in the small intestine. Appendices removed from human beings at operation behave in vitro like strips of colonic tissue from lower animals. Such appendix preparations are of value in the testing of drugs used in human therapeutics (Gunn and Whitelocke, 1914; Kerr Cross, 1924). Within the colon itself the rhythmicity and tonicity is greater caudad than craniad (Alvarez and Starkweather, 1918a; Alvarez and Zimmermann, 1928; Alvarez, 1929; Ascanio and Alvarez, 1929). Alvarez and Starkweather (1918b) claim that there is a metabolic gradient from the cranial to the caudal end of the colon. This gradient is more obvious in herbivores than in carnivores where it may even be reversed.

As is well known, Alvarez seeks to explain the translation of bowel contents caudad by a gradient of gut activity from the oral to the aboral regions of the gut, and he fully elaborates this hypothesis in his two books (1922; 1928). However, not all observations on the large bowel support this theory, due probably to the fact that normally the contents of the colon may move either caudad or craniad.

The decentralized colon of the dog exposed in a saline bath rarely shows signs of activity, although a wave of contraction, arising in the small intestine, may pass for a short distance caudad along the colon (Bayliss and Starling, 1900–1901; Starling, 1910). Distention of a balloon within the colon elicits slow rhythmical contractions of nearly one minute's duration. These waves seem to move caudad. Smaller and briefer contractions are superimposed on these waves. Prolonged variations in tone sometimes occur. "Descending inhibition" is obtained with ease, but "ascending excitation," although present, is difficult to elicit. In the rabbit, under similar conditions, the distal colon shows great activity, "ascending excitation" is obvious, but "descending inhibition" can be demonstrated only with difficulty.

Elliott and Barclay-Smith (1904) studied the movements of the decentralized large bowel of carnivorous, of omnivorous and of herbivorous mammals. They describe three types of movement in the colon.

1. Antiperistaltic waves drive the fluid contents of the colon toward the cecum. From time to time the antiperistaltic waves are replaced by peristaltic waves. Neither the antiperistaltic nor the peristaltic waves of this group are preceded by inhibition of the gut. This type

of contraction, although it may occur anywhere in the colon, is usually confined to the proximal colon, so that the liquid chyme, newly received from the ileum, is passed to and fro over the surface of the colon where absorption occurs. The cecum and proximal colon of herbivora, in the intervals between peristaltic and antiperistaltic contractions, show marked segmentation.

2. When the contents of the colon become firm in consistency, peristaltic waves drive the fecal matter into the terminal portion of the large bowel. These waves are preceded by inhibition of the gut. Usually these peristaltic contractions are seen only in the intermediate and distal colon, but they can occur in the proximal colon.

3. Powerful contraction of the walls of the distal colon evacuates the gut. This movement is accompanied by relaxation of the anal sphincters and is under control of the central nervous system.

Herbivorous animals show well all three types of movement and the regional separation of the movements is sharply defined. Carnivorous animals show only vestiges of the two former types, their short colons, under control of the central nervous system, acting mainly as passive reservoirs for fecal matter.

It is tempting to believe with the older workers that the movements of the first group are "myogenic," and the movements of the second group "neurogenic" in nature. Cannon (1912a; 1912b) has shown, in the decentralized colon of the cat, that the movements of the first group arise from rhythmically pulsating tonus rings. Each pulsation of a tonus ring leads to distention of the colonic walls in the immediate neighbourhood. Such distention elicits a wave of contraction which propagates itself along the colon. When the colonic contents are fluid on both sides of the pulsating ring, waves of contraction pass both craniad and caudad. If, however, the fecal matter is firm caudad to the ring and fluid craniad, the stimulus of distention can occur only proximal to the ring, thus giving rise to the typical antiperistaltic waves of the proximal colon.

The stimulus determining the second type of movement with descending inhibition is obscure. Cannon suggests that, when a tonus ring occurs where the bowel contents are inspissated, the fecal matter is pushed by the muscular contraction, and that this movement of semisolid feces within the colon acts as the stimulus. On the other hand, fluid colonic contents will "give" before a contracting tonus ring while firm fecal matter will not do so. The work of Payne and Poulton (1927), which shows that the sensory disturbance is far greater when the eso-

phageal wall attempts to contract on an unyielding object than when it successfully overcomes a distending force, may bear on this problem.

It is uncertain what part the central nervous system plays in coördinating the various movements of the large bowel. In acute experiments at least, decentralization increases the intrinsic movements without apparent disturbance of coördination (Meltzer and Auer, 1907a, 1907b; Lyman, 1913; Dennig, 1924; Tidmarsh, 1932). The evacuation of the gut contents, however, is seriously disturbed. In animals allowed to recover after destruction of the lumbo-sacral region of the spinal cord, and in man, after injury to the lumbar and sacral regions of the cord, there is a variable period of time during which defecation is most imperfect. Subsequently, however, evacuation of fecal matter occurs at regular intervals, and, although there is no voluntary control of the act, a semblance of defecation sets in. Unfortunately, many workers regard this partial restoration of function as evidence that the distal part of the colon is normally independent of the central nervous system, and that the control of defecation is essentially extra-spinal (Goltz and Ewald, 1896; Lewandowsky and Schultz, 1903; Bálint and Benedict, 1906; Müller, 1902, 1906; Head and Riddoch, 1917; Riddoch, 1917; Hurst, 1919). Other workers deny that true defecation ever occurs after decentralization of the distal colon (Elliott and Barclay-Smith, 1904; Roussay and Rossi, 1908; Matti, 1909; Ten Caté, 1924). Garry (1933b) failed to obtain true defecation responses from the large bowel of decerebrate cats after destruction or anesthetisation of the lumbo-sacral cord. Infrequently, however, under such conditions, vigorous stimulation did elicit an expulsive effort from the colon. Further discussion of this matter may be found in Report 88 of the Special Report Series of the Medical Research Council (1924).

Such conflicting views may be reconciled to some extent. The colon apparently possesses a large degree of autonomy, especially the proximal and intermediate portions. The distal colon, however, seems normally to be in subjection to the central nervous system. When this central control is destroyed, the caudal part of the colon acquires, or more probably regains, the power of independent functioning. Such a conception dispenses with the need for postulating the existence of extra-spinal centres for the act of defecation alone, and explains the variable time which elapses between the destruction of the spinal cord and the appearance of automatic evacuation of the colon.

b. *The ileo-colic and anal sphincters.* After destruction of the spinal cord in the rat and in the rabbit, the ileo-colic sphincter is no longer

effective as the fecal matter forms a continuous column in the proximal portion of the colon and in the distal part of the ileum (Elliott and Barclay-Smith, 1904). On the other hand, Tönnis (1924) obtained in vitro preparations in which the ileo-colic sphincter retained its tone. In oxygenated Tyrode, peristaltic waves drove the contents of the ileum through the sphincter into the colon, but any attempt to pass material from the colon into the ileum led to tonic contraction of the ileo-colic sphincter.

Little is known about the internal anal sphincter. Much work, however, has been carried out on the behaviour of the decentralized external anal sphincter.

Destruction of the lumbo-sacral cord causes dilatation of the anal canal. In the course of time this dilatation becomes less obvious, and, on superficial examination, the anal canal appears to be normally closed. Part of this closure is probably due to the unstriped sphincter, since the inferior mesenteric ganglia exert a tonic influence on the internal anal sphincter (Fuld, 1895; Langley and Anderson, 1895a). Many writers believe that the external anal sphincter also contributes to the closure of the anal canal after cord destruction. Goltz and Ewald (1896), Arloing and Chantre (1897, 1898), Frankl-Hochwart and Frölich (1900), Sherrington (1900), Elliott and Barclay-Smith (1904) and Elliott (1906) all find that the external anal sphincter does not lose its tone after destruction of the lumbo-sacral cord and even after section of the pudendal nerves. In addition, Goltz and Ewald found that, when all the somatic striped musculature of their "cordless" dogs had been replaced by fibrous tissue, the external anal sphincter showed no sign of degeneration and still responded to stimulation by an induced electrical current. Normally, too, the response of the external anal sphincter to stimulation of the pudendal nerves resembles the response from smooth muscle (Arloing and Chantre, 1897, 1898; Frankl-Hochwart and Frölich, 1900). The external sphincter is also supposed to be resistant to the action of curare. Findings such as these have led to the belief that the external anal sphincter is practically independent of connection with the central nervous system and is probably under control of local nerve ganglia.

On the other hand, Gowers (1877), Müller (1902), Benedict (1906) and Matti (1909) believe that the external anal sphincter is paralysed by decentralization. Ten Caté (1922, 1924) examined histologically the external anal sphincter six weeks to two months after pudendal nerve section or after destruction of the sacral cord and found marked degeneration of the muscle fibres. Neither is there any valid support for the

idea that the striped sphincter is wholly resistant to curare, although the dose required to produce paralysis is admittedly large (Langley and Anderson, 1895a; Frankl-Hochwart and Frölich, 1900; Dale, 1906; Ten Caté, 1924; Learmonth and Markowitz, 1929).

It is probable that the confusion which has arisen is due to the mistaken assumption that all striped muscle is qualitatively the same. The external anal sphincter seems to be an extreme example of a "red" muscle with small fibres rich in sarcoplasm. The nature of the muscle fibres themselves may determine the reaction of the muscle to denervation and to curare.

4. THE LARGE BOWEL IN THE INTACT ANIMAL. a. *The colon.* In an intact *lower animal* the movements of the proximal and intermediate regions of the colon are similar to those already described in the excised and decentralized large bowel. Cannon (1902), using opaque meals and enemata, found in cats that entrance of chyme from the ileum initiates strong antiperistaltic waves in the proximal colon. These waves occur in bursts of approximately five minutes' duration and at intervals of from a quarter to half an hour. Segmentation occurs infrequently and broad constrictions sometimes rhythmically knead the colonic contents. About the middle of the colon tonic constrictions appear and nip off a portion of the fecal matter. These constrictions travel slowly caudad driving the feces before them. In two cases Cannon observed a massive wave of contraction which drove the entire contents of the proximal colon into the distal colon. The antiperistaltic movements of the colon are easily inhibited by fear and by distress but continue actively during sleep.

Defecation is usually initiated by a powerful wave of contraction which arises about the middle of the colon, the large bowel shortens, even to half its resting length, and the recto-coccygeus pulls the colon toward the pelvis. Associated with these movements is the characteristic defecation posture, contraction of the diaphragm and of the abdominal muscles and relaxation of the anal sphincters. In cats the proximal colon is not usually emptied in defecation. Lenz (1924), observing the cat's colon through an abdominal window, describes similar movements. In the dog the behaviour of the colon is essentially the same as in the cat but defecation may empty the entire large bowel (Barcroft and Steggerda, 1932).

In the large cecum of the rabbit peristaltic waves alternate with antiperistaltic waves. In the proximal colon the antiperistaltic waves are two to three times as frequent as the peristaltic waves and the haustra

show continuous movement. All such movements are inhibited by pain, by fear and by anesthesia (Meltzer and Auer, 1907b; Zondek, 1921). In the rabbit and in the guinea pig the cecal movements lead to very complete mixing of the bowel contents, but in the rat and in the cat little or no mixing occurs (Basler, 1909).

Within recent years many workers have returned to the use of recording balloons introduced through colostomies or through the anus (Welch and Plant, 1926; Plant and Miller, 1926; Quigley and Solomon, 1930; Templeton and Bollens, 1929–30; Templeton and Lawson, 1931; Lawson and Templeton, 1931, 1932; Young, Rice and Steinhaus, 1931). Records obtained in this way from unanesthetised dogs usually show three types of movement—small rhythmical waves occurring several times a minute, larger waves at roughly minute intervals and slow tone variations three or four times in the hour.

The movements of the large bowel are influenced by activity in other regions of the gut. Eating food increases colonic activity within a few minutes (Welch and Plant, 1926; Hinrichsen and Ivy, 1931; Barcroft and Steggerda, 1932). Welch and Plant failed to get this response when food was introduced directly into the stomach through a fistula, but Hinrichsen and Ivy believe that it is the actual presence of the food within the stomach and the duodenum which is responsible for the increased activity of the colon. There is an association between defecation and feeding in birds (Reed and Reed, 1925).

For our knowledge of the movements of the large bowel in *man* we are deeply indebted to Hurst (1907, 1908, 1909a, 1909b, 1919). Antiperistalsis, somewhat unexpectedly in view of the structure of the human colon, is not an obvious phenomenon. When visible, the waves are shallow and start in the transverse colon close to the hepatic flexure. The haustra of the cecum and of the ascending colon may show frequent small contractions. Three or four times a day, a massive peristaltic wave drives the contents of the proximal colon bodily into the distal colon. Before and during the movement the the haustral segmentation disappears. Holzknecht (1909) was the first to detect this mass movement in the human colon. In man the rectum is normally empty and fecal matter accumulates proximal to the pelvi-rectal flexure (O'Beirne, 1833). As a result of one of the infrequent massive peristaltic waves fecal matter enters the rectum and the call to defecation becomes clamant. In the act of defecation itself strong peristaltic contractions occur throughout cecum and colon, the intra-abdominal pressure is raised by contraction of the diaphragm and of the abdominal muscles,

and the feces are forced through the relaxed sphincters of the anal canal. The levator ani aids defecation by pulling the anal canal craniad over the extruding fecal mass and the pubo-rectal fibres of the levator ani, by constricting the rectum, clear out the last traces of fecal matter. Although the entire large bowel is active in defecation fecal matter is normally removed only from the distal part of the colon.

Mass peristalsis seems to be the usual method of conveying fecal matter in man into the distal colon, yet Fischl and Porges (1911), in a somewhat abnormal case, observed very deliberate movement caudad of isolated fecal masses from the transverse into the distal colon. Such movement reminds one irresistibly of the transport of fecal pellets in the distal colon of herbivora. Whether or not this is a normal state of affairs. there seems to be no justification for regarding the movements of the human large bowel as unique. Case (1915), indeed, goes so far as to state that he was able to confirm in human beings Cannon's observations on cats "almost to the minutest detail."

Post mortem in man, fecal matter is usually confined to the cecum and to the ascending and transverse colon (Roith, 1903, 1909, 1913). This disposition of the fecal matter is probably due to antiperistalsis and to the normally high tone of the distal colon. Singer and Holzknecht (1911), Westphal (1927) and others believe with Keith that exaggeration of this tone often leads to a common form of obstipation.

Records of the movements of the human colon have frequently been obtained by means of balloons introduced through colostomies or through the anus (Ganter and Stattmüller, 1924; Weitz and Vollers, 1926; Alvarez, 1929). There are small waves with a frequency of 4 to 5 per minute and large waves at roughly minute intervals. The colon as a whole is comparatively refractory to distention and the rectum proper shows practically no movement.

As in lower animals, eating food increases the activity of the human colon and frequently initiates a mass peristaltic contraction leading to defecation. This response was first noted by Macewen (1904) in patients with cecal fistulae. Subsequently the name gastro-colic reflex was applied by Hertz (1909a, 1909b) and by Hertz and Newton (1913). Other stimuli affect the activity of the colon. Exposure of the skin of the thorax to cold air increases colonic activity, but a hot water bag applied to the skin of the abdomen, a cold foot bath and drinking cold water inhibit colonic movements. An ice bag, a hot foot bath and hot tea are not so effective (Weitz and Vollers, 1927).

b. *The ileo-colic sphincter.* In 1898 Grützner claimed that, both in

man and in lower animals, material in the large bowel may enter the ileum and even reach the stomach. Grützner's earlier experiments were subjected to severe criticism, but modern opinion tends to accept the validity of his conclusions. In a certain proportion of cases the ileo-colic valve is incompetent (Klee, 1912; Hertz, 1913; Cole, 1914; Case, 1915; Hurst, 1919). No human ailment can be attributed with certainty to this condition. It is significant that Alvarez and Mahoney (1924) believe that the proper function of the ileo-colic sphincter is not to act as a one-way valve but is to prevent too rapid emptying of the ileum by interrupting peristaltic rushes, which, if unchecked, would lead to premature defecation.

Like the colon the ileo-colic sphincter is influenced by the condition of the rest of the gut. In a starving animal the ileo-colic sphincter is patulous; when the animal is fed the sphincter closes (Hannes, 1920). Distention of the proximal colon causes contraction of the ileo-colic sphincter (Heile, 1905; Tönnis, 1924). Within a few minutes of taking food into the stomach, not only the proximal colon but also the distal ileum shows increased activity. The ileo-colic sphincter relaxes intermittently and allows spurts of fluid to escape into the colon (Short, 1919; Hinrichsen and Ivy, 1931). Absence of such responses may aid in the diagnosis of pathological processes in the region of the ileo-cecal sphincter (Maclean, 1932).

5. The effect of injury to the central nervous system. Undoubtedly the act of defecation is normally under voluntary control, but there are subsidiary centres in the neural axis. Stimulation of the medulla close to the vomiting centre causes defecation (Hatcher and Weiss, 1923; Koppanyi, 1930–31). There must also be a defecation centre in the lumbo-sacral region of the spinal cord of animals since cord transection in the lower thoracic region has no effect on the essential act of defecation (Sherrington, 1900; Cannon, 1911; Klee, 1912, 1913; Garry, 1933b).

Until comparatively recent times, clinicians taught that all transverse lesions of the spinal cord in man lead to permanent disturbance in defecation. Head and Riddoch (1917) and Riddoch (1917), however, have vindicated the findings of physiologists, even where man is concerned. In man spinal shock lasts for several weeks after cord transection and, during this period, defecation is in abeyance. Thereafter the normal coördinated act of defecation returns whenever the proper stimulus is applied. The effect of cord destruction has already been discussed.

Transection of the neural axis craniad to the lumbo-sacral region in-

creases the tonicity of the external anal sphincter and distention of the anal canal now elicits rhythmical contraction and relaxation of the striped sphincter at a rate of 20 to 25 beats per minute (Goltz and Freusberg, 1874; Ott, 1879; Langley and Anderson, 1894a). This phenomenon occurs after division of the crura cerebri (Ott, 1880–82). Apparently inhibitory impulses, arising craniad to the hind brain, act on a centre in the lumbo-sacral cord.

6. THE EFFECT OF SECTION OF VISCERAL OUTFLOWS. Unfortunately there are few observations on the effect of simple division of nervous outflows. Usually the nerve section is incidental to some other manipulation such as nerve stimulation.

a. *The thoracico-lumbar outflow: The colon.* The majority of workers find that division of the lumbar outflow leads to increased colonic activity (Bayliss and Starling, 1900–1901; Elliott and Barclay-Smith, 1904; Magnus, 1906; Lehmann, 1913; Tönnis, 1924; Learmonth and Markowitz, 1930; McCullagh, McFadden and Milroy, 1930). Defecation, too, can be elicited with greater ease after division of the lumbar outflow (Garry, 1933b). Spinal anesthesia very definitely increases the movements of the colon (Arai, 1922a; Markowitz and Campbell, 1927; Garry, 1933a). Such results suggest that the dominant influence of the central nervous system on the large bowel is inhibitory. The inhibitory impulses arise locally in the lumbo-sacral region of the cord, pass by the spinal rami to the inferior mesenteric ganglia and thence by the lumbar colonic nerves to the gut. The hypogastric nerves do not play an obvious part in the transmission of inhibitory impulses to the colon. The inferior mesenteric ganglia seem to be able to act as extra-spinal centres for inhibition (Garry, 1933a).

Wade and Royle (1927) suggest that the thoracico-lumbar outflow to the gut, on analogy with the hypothetical action of the same outflow on the tone of skeletal muscle, controls the postural tone of the bowel. Learmonth and Markowitz (1930) make a similar suggestion.

In opposition to the above findings are the results of Ken Kuré and his collaborators (1931) who find that section of the thoracico-lumbar outflow causes loss of tone in the distal part of the small bowel and in the proximal colon. Such findings are explained by the presence of spinal parasympathetic fibres.

The ileo-colic sphincter. Division of the thoracico-lumbar outflow causes relaxation of the ileo-colic sphincter (Elliott, 1904; Hinrichsen and Ivy, 1931). Lyman (1913) and Tönnis (1924) find that such relaxation is only temporary.

The internal anal sphincter. Division of the lumbar outflow causes relaxation of the internal anal sphincter. Removal of the inferior mesenteric ganglia accentuates this relaxation (Langley and Anderson, 1895a; Barrington, 1915).

One is apt to wonder how permanent is the effect of interruption of the lumbar outflow. In experiments where recovery of the animal occurs the effect seems to be transient. In addition, in acute experiments the results of nerve section may not indicate the normal flow of impulses to the viscera. The operative interference necessary to expose the visceral nerves undoubtedly plays a part in producing reflexly the very inhibition which subsequent nerve section will remove. Cannon, Newton, Bright, Menkin and Moore (1929) find that exclusion of all thoracico-lumbar impulses in cats leads to "nothing abnormal—in the activities of the digestive canal." As a result, Cannon (1930a, 1930b) believes that the "sympathetic" system is called into action only in an emergency. May the interference in an acute experiment not be an emergency, so that the results of nerve section give a wholly spurious idea of the normal functioning of the thoracico-lumbar outflow?

b. *The bulbo-sacral outflow: The colon.* It is probable that the disturbance in colonic function, which results from destruction of the sacral region of the cord, is due to abolition of impulses in the sacral outflow. As a result of section of the pelvic nerves in cats and in dogs the movements of the distal colon become weak and sluggish, and, after an interval of several weeks, megacolon may develop (Elliott, 1906; Cannon, 1911b; Adamson and Aird, 1932). The presence of a motor outflow in the pelvic nerves is also suggested by the work of Learmonth and Markowitz (1930) and of Young, Rice and Steinhaus (1931). Even in acute experiments, section of the pelvic nerves causes loss of tone in the colonic walls, and defecation responses can no longer be elicited (Garry, 1933a, 1933b). The motor impulses for the colon arise mainly craniad to the lumbo-sacral cord. The pelvic nerves not only convey efferent impulses to the colon but also afferent impulses from the colon. The innervation of the urinary bladder is similar (Barrington, 1915, 1921).

The effect of vagal section on large bowel movements is thoroughly obscure. At the most one would expect the results to show only in the proximal colon. Koennecke (1922) reviews such information as we possess.

The ileo-colic and internal anal sphincters. There is no satisfactory evidence that section of the bulbo-sacral outflow affects the ileo-colic sphincter. Somewhat unexpectedly, division of the pelvic nerves, or

of the posterior roots of the sacral nerves, seems to cause relaxation of the internal anal sphincter (Frankl-Hochwart and Frölich, 1900; Merzbacher, 1902; Matti, 1909; Learmonth, 1926–27).

c. *Section of the thoracico-lumbar outflow in man as a therapeutic measure.* If one disregards Ken Kuré's work and concludes that impulses passing by the lumbar outflow inhibit the large bowel and cause contraction of the internal anal sphincter in man, then partial or total interruption of this outflow is indicated in pathological cases where it can be shown that the defect is due to actual or to relative overaction of this outflow. Fraser (1926) believes that the innervation of the gut through the thoracico-lumbar outflow precedes developmentally the innervation through the bulbo-sacral outflow. Thus, in the early days of life, nerve impulses leading to inhibition of the colon and to contraction of the internal anal sphincter are in the ascendant. This may be the cause of Hirschsprung's disease.

On the other hand, Hurst (1925) and Hurst and Gaymer-Jones (1930) believe that the essential pathological process in Hirschsprung's disease is achalasia, an absence of relaxation, at the internal anal sphincter, less commonly at the pelvi-rectal sphincter. This absence of relaxation is supposed to be due to degeneration of Auerbach's plexus caused initially by inflammation of the mucous membrane. Cameron (1928) has shown such degeneration histologically.

Whether or not section of the lumbar outflow attacks the primary pathological condition, the results of surgical intervention in man are gratifying. Bartle (1926), Wade and Royle (1927), Judd and Adson (1928), Wade (1930) and Royle (1930) recommend excision of the lumbar sympathetic chains and ganglia, or division of the medially running strands of the chains on one or on both sides. Rankin and Learmonth (1930, 1932) prefer to cut the inferior mesenteric and presacral nerves.

The possibility of similar surgical intervention in cases of intractable constipation is attractive. Royle (Wade and Royle, 1927) noted that lumbar sympathetic ramisection in cases of spastic paralysis of the lower limbs causes marked alleviation of the constipation so common in such patients. A priori, not all cases of obstipation should yield to section of the lumbar outflow. According to clinicians there is a form of constipation due to hypertonicity or hyperactivity of the distal colon. Fecal matter is either prevented from reaching the rectum or is actively forced craniad. Such activity is presumably due to actual or relative dominance of the sacral outflow. In such cases division of the lumbar outflow should only aggravate the condition.

7. THE EFFECT OF STIMULATION OF VISCERAL OUTFLOWS. In striking contrast to the poverty of information concerning the effect of simple division of the outflows to the large bowel is the wealth of data accumulated by stimulation of the outflows. The older workers, too, spent much time investigating the effect of stimulating the cortex cerebri and other parts of the central nervous system. It is very difficult to assess the importance of their findings.

a. *The thoracico-lumbar outflow: The colon.* It is part of the dogma of physiology that stimulation of the "sympathetic" outflow inhibits gut movements. At one time it was believed that the lumbar outflow causes contraction of the circular coat of the colon and relaxation of the longitudinal coat and of the rectococcygeus (Gaskell, 1886; Fellner, 1894; Winkler, 1904). Starling (1902, 1910) attacked this theory viciously, and only now is it showing signs of recovery. In the observations of the older and of the very recent workers it is usual to find that stimulation of some part of the lumbar outflow, during some phase of the experiment, has a motor effect on the large bowel (Bechterew and Mislawski, 1889; Langley, 1891; Langley and Anderson, 1895a; Hinrichsen and Ivy, 1931; Ken Kuré, 1931). The initial tone of the gut walls may also affect the nature of the response to stimulation of the lumbar outflow; if the tone is high, as it probably will be, due to severance of the lumbar outflow preparatory to stimulation, inhibition occurs; if the tone is low, contraction occurs (Boehm, 1913; Carlson, 1930).

The ileo-colic sphincter. Stimulation of the lumbar outflow causes contraction of the ileo-colic sphincter (Elliott, 1904; Hinrichsen and Ivy, 1931). Katz and Winkler (1904) find the opposite effect.

The internal anal sphincter. In the cat and in the dog stimulation of the lumbar outflow causes contraction of the internal anal sphincter (Langley and Anderson, 1894a; 1895a; Elliott, 1905; Dale, 1906; Lehmann, 1913; Learmonth and Markowitz, 1929). So long as the opposite hypogastric nerve or the lumbar colonic nerves are intact, stimulation of the central end of one hypogastric nerve will lead to contraction of the internal anal sphincter even after interruption of all connection between the inferior mesenteric ganglia and the spinal cord. This is one of the best known examples of an axon (Sokownin) reflex.

In the rabbit stimulation of the lumbar outflow leads to dilatation of the internal anal sphincter (Langley and Anderson, 1895a); and Dale (1906) concludes that there are inhibitory fibres for the internal anal sphincter in the hypogastric nerves of the cat but that they are usually masked by the presence of dominant motor fibres. Frankl-Hochwart

and Frölich (1900) claim that stimulation of the lumbar outflow inhibits the internal anal sphincter in dogs. Their recording method, however, is open to criticism.

The action of adrenaline. Adrenaline, in the dosage usually employed in experimental work, inhibits colonic movements and lowers the tone of the gut walls (Langley, 1901–02; Magnus, 1903, 1905, 1908; Elliott 1905; Ganter and Stattmüller, 1924). In vitro the action is similar (Currie and Henderson, 1926; Gunn and Whitelocke, 1914; Suckow and Burget, 1929). In some few cases Magnus found that adrenaline has a motor action on the gut, and very recently Templeton and Lawson (1932) have obtained a diphasic augmentation of colonic movements in dogs by giving adrenaline intravenously. The first phase is due to contraction of the circular coat and to relaxation of the longitudinal coat of the colon; the second phase is due to relaxation of the circular coat and to contraction of the longitudinal coat. In response to drugs the first phase is "sympathetic" and the second phase "parasympathetic" in nature.

The ileo-colic sphincter, in vivo and in vitro, is closed by adrenaline (Elliott, 1904; Kuroda, 1917; Hinrichsen and Ivy, 1931).

Adrenaline causes contraction of the internal anal sphincter (Elliott, 1905; Dale, 1906; Learmonth and Markowitz, 1929). Langley (1901–02) found that adrenaline inhibits the internal anal sphincter of the rabbit.

b. *The bulbo-sacral outflow: The colon.* In keeping with von Basch's theory of crossed innervation, Gaskell (1886), Fellner (1894) and Winkler (1904) find that stimulation of the pelvic nerves causes contraction of the longitudinal coat of the distal colon with simultaneous relaxation of the circular coat. Practically all other workers find that stimulation of the sacral outflow leads to contraction of both coats of the colon. The effect is most pronounced in the distal colon, but extends to the proximal colon in the form of augmentation of antiperistalsis (Elliott and Barclay-Smith, 1904) or of tonic contraction (Boehm, 1913). The pelvic nerves are the motor nerves to the colon of monkeys (Carlson, 1930). By stimulation of the sacral outflow Langley and Anderson (1895a) caused contraction of the distal colon in rabbits, but infrequently transient relaxation occurred.

Langley (1903), reviewing the innervation of the intestinal tract, somewhat tentatively, and mainly on theoretical grounds, suggests that vagus control extends to the proximal and intermediate colon. In the rabbit, stimulation of the peripheral end of one vagus nerve causes tonic

contraction of the cecum (Meltzer and Auer, 1907b). According to Bechterew and Mislawski (1889), Winkler (1904) and Boehm (1912, 1913), stimulation of the vagus has an undoubted motor effect on the proximal colon, but Bayliss and Starling (1900–01), Starling (1910) and Klee (1912) deny this. Carlson (1930) believes that the vagus is the motor nerve to the proximal colon in monkeys but not in dogs. How far this effect of the vagus on the colon is due to extension of a motor effect from the small bowel is debatable. Boehm believes that the effects he observed were not due to such extension.

The ileo-colic sphincter. It is doubtful if stimulation of the bulbo-sacral outflow ever affects directly the ileo-colic sphincter (Elliott, 1904; Katz and Winkler, 1904; Trendelenburg, 1927; Hinrichsen and Ivy, 1931).

The internal anal sphincter. On theory one would expect stimulation of the sacral outflow to cause relaxation of the internal sphincter. So far the results obtained in the cat and in the dog are equivocal (Langley and Anderson, 1895a; Elliott, 1905; Lehmann, 1913). In the dog Frankl-Hochwart and Frölich (1900) obtained contraction, and Langley (1911) caused definite relaxation of the internal anal sphincter in the rabbit by stimulation of the roots of the sacral nerves.

The action of choline derivatives. Acetylcholine given hypodermically or intravenously causes intense gut activity (Dale, 1914, 1915). Le Heux (1919, 1924) finds that choline, however powerful its action on the stomach and small intestine, has little action on the large bowel. Arai (1922b), on the other hand, finds that choline HCl given intravenously has an equally powerful action on the small and large intestine leading to normal defecation. In therapeutics, choline, given intravenously, is recommended to promote normal gut activity where there is atonic constipation or reflex inhibition of the gut (Magnus, 1925; Klee and Grossmann, 1925).

8. THE REFLEXES CONTROLLING DEFECATION. The stimulus eliciting defecation is normally applied to the caudal part of the large bowel (Zimmermann, 1909; Hertz, 1911). In certain cases, stimuli applied outside the colon, for example to the central ends of the vagi and of various somatic nerves, may elicit defecation (Lehmann, 1913; Koppanyi, 1930–31). Such stimuli probably send impulses directly to the defecation centre in the medulla, but it is very doubtful if this is a normal occurrence.

It is convenient to recognise two stages in the process of defecation (Garry, 1933b). An effective stimulus to the caudal end of the colon

leads to contraction of the colon and to relaxation of the anal sphincters. The afferent pathway from the gut, and the efferent pathways to the colon and to the internal anal sphincter, are in the pelvic nerves. The response of the colon, unlike the corresponding first micturition reflex, is not abolished by transection of the spinal cord in the lower thoracic region. The response of the internal anal sphincter resembles in all ways the sixth micturition reflex described by Barrington in 1931. The efferent pathway for the external anal sphincter must be in the pudendal nerves, the response corresponding to Barrington's fifth micturition reflex.

The passage of fecal matter through the anal canal acts as a potent stimulus augmenting the dilatation of the anal canal and the contraction of the colon. Probably the afferent impulses concerned pass both by the pelvic and the pudendal nerves. No part of the cerebro-spinal axis craniad to the lumbo-sacral cord is required for these responses. In this respect the response of the colon to stimulation of the anal canal is unlike Barrington's second micturition reflex. Stimulation of the anal canal will cause dilatation of the anal canal after section of the pudendal nerves, the afferent and efferent pathways being in the pelvic nerves. The pathways for the corresponding response of the urethra are in the pudendal nerves.

CONCLUSION AND SUMMARY

The present is an iconoclastic age in physiology. The cut and dried, the systematised hypotheses of the end of the nineteenth and the beginning of the twentieth century are being called in question. There is less glib transference of results from one type of animal to another. The need for morphological knowledge as a basis for physiological experimentation is being more fully realised. This is well exemplified in the physiology of the large bowel where the form and function are indissolubly connected. Differences in morphology make the transference of results from lower animals to man peculiarly difficult.

More than ever before the limitations of experimentation are being realised. Without knowledge of the methods employed results are valueless. Our present conception of the physiology of the large bowel requires to be examined in the light of the technique of the past. The results obtained are valid only for the conditions of the experiment and should with the greatest caution be embodied in our conception of the normal functioning of the intact animal.

Results obtained by the in vitro technique are surprisingly free from

objection because the factors concerned are easily controlled and the limitations of the method are obvious. In vitro, the large bowel, although rather sluggish in behaviour, shows the same autonomous powers as the rest of the intestinal tract. There seem to be two definite types of movement. It is not justifiable to call one peristalsis and the other antiperistalsis, and the difference between them is not yet elucidated.

Simple observations on the large bowel in the intact animal are free from objection and show that the behaviour in lower animals is essentially similar to that in man, although the details may be modified by the form of the gut.

Injury to the central nervous system shows the large degree of autonomy possessed by the large bowel, even complete decentralization causing only temporary disturbance in evacuation of fecal matter. This fact does not justify the assumption that the act of defecation is normally controlled by extra-spinal centres. Probably the distal colon is more intimately under control of the central nervous system than other parts of the intestinal tract.

Modern work shows that the results obtained by nerve section and by nerve stimulation, especially by nerve stimulation, should be accepted with reserve. The hypothesis of "sympathetic" and "parasympathetic" mutually antagonistic control of the colon is suspect. Even the results of past workers, on which this theory is founded, do not justify wholehearted acceptance of this conception. It is unlikely that the results are due to indeterminancy in the animal body. They more likely depend on inadequate knowledge of the microscopic anatomy of the nervous outflows to the gut, and on the crudity of experimental methods. The explanation of the experimental results waits on more accurate knowledge of the histology of the innervation of the large bowel.

The reflex control of defecation is seen to resemble closely the reflex control of micturition, although the dependence on the central nervous system is not so marked.

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THE COMPARATIVE PHYSIOLOGY OF THE KIDNEY IN RELATION TO THEORIES OF RENAL SECRETION

E. K. MARSHALL, JR.

The Department of Pharmacology and Experimental Therapeutics, Johns Hopkins School of Medicine, Baltimore, Maryland

It is interesting to note that Bowman, whose name will always be associated with the physiology of the kidney, early recognized the value of comparative anatomical and physiological studies in this field; it is equally interesting to see how little has been accomplished since then in a systematic way in approaching the problems of renal secretion from this standpoint. Bowman (1842) in his classical paper on the kidney presents many observations on the comparative anatomical side; he even attempts a quantitative comparative study by measuring the diameter of the malpighian bodies and tubules in "several orders of animals." He noted the correspondence between the size of the renal corpuscles and the length of the tubules, and emphasized the need of further study in this direction. After stating his theory of urine secretion and the morphological evidence upon which it is based, Bowman writes: "Parallel lines of inquiry into the anatomical varieties of the malpighian bodies and uriniferous tubes, and into the chemistry of their secretion, in different tribes of animals and in various stages of their development, could scarcely fail either to confirm or to confute what has now been advanced."

Hüfner, a pupil of Ludwig, in 1866 published an inaugural dissertation on the comparative anatomy and physiology of the urinary tubules. Using the maceration and injection technique, he studied the renal tubules of several classes of vertebrates and gave measurements of the size of the renal corpuscles and tubules. His plate picturing tubules from the fish, frog, turtle, bird and mammal is extremely instructive. Hüfner believed that the same four segments are present in the tubules of all vertebrates but that their diameter and length vary greatly. He attempted to correlate his anatomical findings with what little was then known about the function of the different kidneys (mainly water excretion).

It may well be that the subsequent neglect of this important method of comparative physiology in attacking the problems of renal secretion

is one of the main reasons that this field is still in such an unsettled state. It is certain that the indiscriminate treatment in the same way of results obtained on widely varying classes of vertebrates without considering the qualitative and quantitative differences in the morphology of the kidneys of the different classes can lead only to confusion. A proper evaluation of experiments on the lower animals in their bearing on human kidney function can be made only from a broad outlook on the whole vertebrate phylum.

A great advantage of the method of comparative physiology in this field is that the disturbing effects of anesthesia and operative procedures can frequently be eliminated without sacrificing the control of experimental variables. The two general methods which have been used in attacking the problems of urinary secretion are: 1, the simplification of the conditions and variables by the use of acute experiments on anesthetized and operated animals or "pieces of animals" containing the renal organ, and 2, a comparison of the changes in blood plasma with the quantity and composition of the urine under different conditions. The first method has the great advantage of the control of variables so necessary in dealing with a complex organ like the kidney, but on the other hand we do not know how far we are justified in transferring results obtained under the abnormal conditions of such experiments to the intact normal animal. The second method does not suffer from this objection, since the experiments can be done on normal, unanesthetized animals, but it has not aided greatly in solving one of the main problems involved in kidney function, the nature of the processes occurring in the morphologically different parts of the renal tubule. By utilizing this second method in a comparative way, the advantages of the two may be combined. A comparative study of the morphology and function of the renal unit as it has been gradually evolved from a simpler to a more complex structure should prove particularly valuable in eliminating the disturbing effects of anesthesia and operative procedures; such a study could be carried out on animals normal in every way so that the only modifications of the experimental conditions would be such as have been established by nature herself.

Structure of renal tubule in different vertebrates. We can consider the mammalian renal tubule as made up of five morphologically distinct segments: 1, the renal corpuscle; 2, the proximal convoluted tubule with medullary segment; 3, the thin part of Henle's loop lined with pavement epithelium; 4, the distal convoluted tubule—from the region where the pavement epithelium of the loop ceases to the initial collecting tubule;

and 5, the initial collecting tubule (Huber, 1909–10).[1] These same segments may be found in some of the lower vertebrates except that segment 3 is absent or replaced by a short tubular segment of small diameter and characteristic ciliated epithelium, which has been designated the intermediate segment. A ciliated neck segment following the renal corpuscle is also present in many of the lower vertebrates.

In table 1 are collected data on the sizes of the different segments occurring in the renal tubule of vertebrates. The measurements are all in millimeters, the length being given first in each case, the width second; blank spaces indicate that the segment does not occur in that kidney. The renal tubules of the different classes of vertebrates are presented in figure 1 in a schematic manner.

The data for the table and drawing were obtained as follows. For tubule *1*, the data of Conel (1917) on *Bdellostoma* and *Myxine* were used. The data for tubule 2 are taken from the observations of Brocea (1906) and Nash (1931) on the skate (*Raia stabulaforis*). For tubule 3 the observations of Nash (1931) and Defrise (1932) on the kidney of the sculpin (*Myoxocephalus octodecimspinosus*) were used. As a basis for tubule 4 I have made measurements from the drawings of an unpublished reconstruction of the nephron of the catfish (*Ameriurus nebulosus*) kindly loaned me by Dr. A. L. Grafflin.[2] For tubule 5 the data of Marshall (1929) and Grafflin (1931) on the kidney of the toad fish (*Opsanus tau*) were used.

The length of the tubule of teleosts varies greatly in different species and to some extent in the same kidney. Thus, the total length of the tubule of the sculpin is about 6 to 8 mm., while that of the cod may be less than 2 mm.; the toadfish

[1] It is possible that some of these segments of the mammalian nephron may be subdivided by future work. Suzuki (1912) distinguishes three sections of the proximal, and Defrise informs me that he has evidence of two types of cells in the proximal segment of the mammal. Edwards and Schnitter (1933) divide the proximal segments of many fish into two parts. At present, it seems best to regard the proximal convoluted segment as a single unit until further research definitely clarifies the matter.

[2] Policard and Mawas (1906) describe the nephron of three fresh water teleosts as made up of glomerulus, proximal segment, distal segment, and collecting tubule. Edwards and Schnitter (1933) have recently described a long ciliated intermediate segment in addition to the above segments in the tubule of the fresh water goldfish (*Carassius auratus*) and a tropical fresh water fish, *Danio malabaricus*. I have here taken Grafflin's reconstruction of the catfish nephron as the basis for my measurements and figure, although in a recent publication (Burgess, Harvey and Marshall, 1933) the nephron of the fresh water fish was given without intermediate segment according to Policard and Mawas' description. I wish to thank Dr. A. L. Grafflin for his kindness in making this reconstruction and allowing me to use it for my measurements and drawing before its publication. Obviously, more careful anatomical work is needed on the nephrons of the kidneys of different species of fish.

TABLE 1

Measurements of segments of renal tubule of vertebrates

NUMBER IN FIGURE	CLASS	SPECIES	RENAL CORPUSCLE	NECK SEGMENT	PROXIMAL CONVOLUTED SEGMENT	INTERMEDIATE SEGMENT	THIN PART OF HENLE'S LOOP	DISTAL CONVOLUTED SEGMENT	INITIAL COLLECTING TUBULE
1	Cyclostome	*Bdellostoma stouti*	0.630 × 0.500	0.400 × 0.050	0.75 × 0.350				
2	Elasmobranch	*Raia stabulaforis**	0.500 × 0.350	0.050 × 0.040	56.00 × 0.120				1.00 × 0.040
3	Teleost	*Myoxocephalus octodecimspinosus*	0.125 × 0.100	0.035 × 0.015	6.06 × 0.055				0.50 × 0.050
4	Teleost	*Ameriurus nebulosus*	0.088 × 0.080	0.108 × 0.025	1.23 × 0.050	0.07 × 0.030		1.13 × 0.035	0.20 × 0.030
5	Teleost	*Opsanus tau*			4.27 × 0.070				0.47 × 0.070
6	Amphibian	*Rana catesbiana*	0.125 × 0.100	0.100 × 0.030	3.80 × 0.075	0.20 × 0.022		2.50 × 0.030	0.70 × 0.028
7	Reptile	*Chrysemys marginata*	0.055 × 0.055	0.025 × 0.020	1.40 × 0.063	0.30 × 0.025		1.00 × 0.038	0.50 × 0.030
8	Bird	*Gallus domesticus*	0.075 × 0.065		3.60 × 0.063	1.20 × 0.030		2.20 × 0.040	1.00 × 0.038
9	Bird	*Gallus domesticus*	0.110 × 0.100		7.00 × 0.063		1.70 × 0.018	4.10 × 0.036	1.20 × 0.038
10	Mammal	*Lepus cuniculus*	0.123 × 0.095		6.90 × 0.036		1.20 × 0.014	5.75 × 0.025	1.00 × 0.020
11	Mammal	*Lepus cuniculus*	0.133 × 0.102		6.90 × 0.035		12.30 × 0.014	4.35 × 0.025	1.00 × 0.020

* The proximal convoluted segment is intercalated between two other segments which are not represented in other vertebrates. The first of these measured 7.00 × 0.050 and the second, 28.00 × 0.050.

tubule is about 4 mm. long, while that of the goosefish may exceed 10 mm. in length. Still other types of tubules than those represented may be found among the heterogeneous group of teleosts. For instance Verne (1921–22) and Edwards (1928) find both proximal and distal segments in the aglomerular nephron of the pipe fish and Edwards (1929) states that a long ciliated segment occurs before the brush border segment in the kidney of *Muraena helena*.

The values for tubules *6* and *7* are taken from reconstructions of tubules of the frog (*Rana catesbiana*) and the turtle (*Chrysemys marginata*) described by Huber (1917); for tubules *8* and *9*, from data for the chicken (*Gallus domesticus*) given by

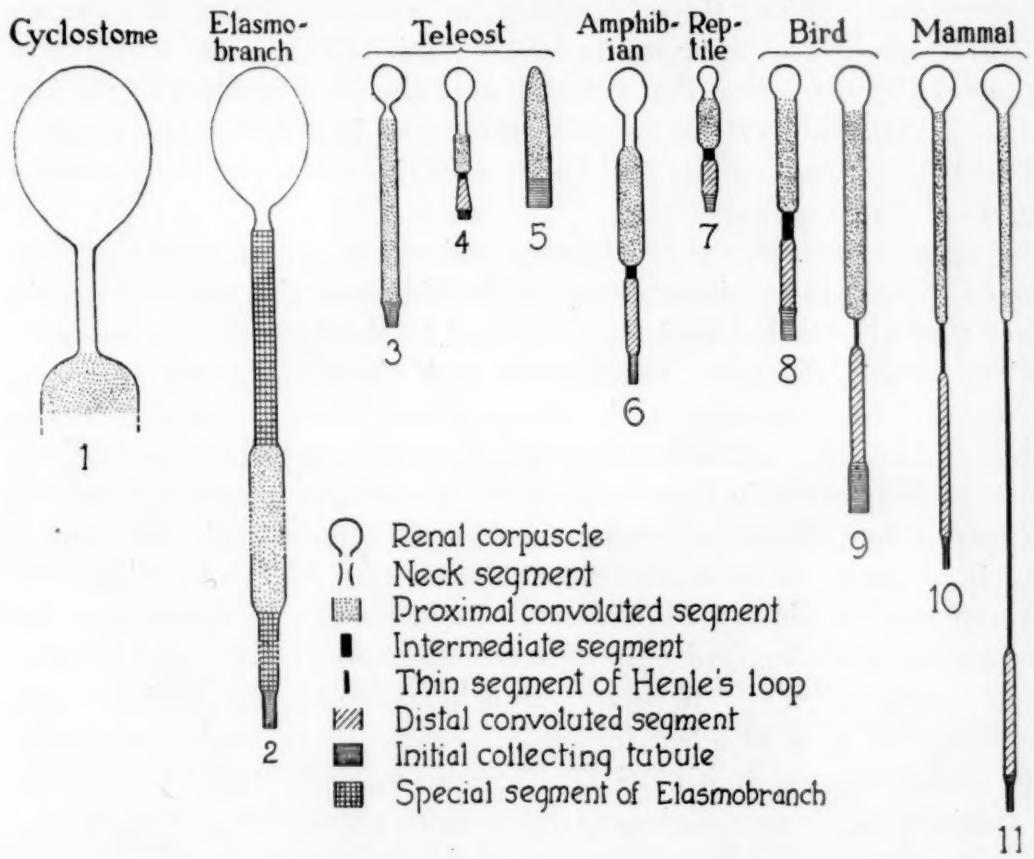

Fig. 1. Schematic representation of nephron of different vertebrates

Huber (1917) and Feldotto (1929); and for tubules *10* and *11* from the data of Peter (1909) for the rabbit (*Lepus cuniculus*). Tubule *8* represents the reptilian type present in the bird, while *9* is the less frequent mammalian type. Tubules *10* and *11* are intended to represent mammalian tubules with short and long medullary loops. Sufficient data are not available for inclusion of tubules from the dipnoan (lungfish) or ganoid.

The measurements are at best only rough approximations and are not exactly comparable as some are taken from macerated preparations (which may shrink or swell) and others from reconstructions from serial sections (where shrinkage occurs). However, since variations in the measurements of tubules in different

species and even in the same kidney are so great, this is of minor importance at present. In figure 1, the drawings are roughly comparable as to scale in the whole series for any particular segment but not for the entire tubule. As seen in table 1, the elasmobranch tubule is even longer than a mammalian tubule. Its extraordinary length, as compared with that of the tubule of other classes, has made it impossible to represent it in full in figure 1.

It is evident that the proximal convoluted segment, as judged by the presence of a brush border, is the only one occurring in all classes of vertebrates; each of the other segments, including the renal corpuscle with its glomerulus, is absent in one or another of the lower vertebrates. It would appear, then, that correlation of the total function of the kidneys of different vertebrates with their morphological structure offers a method of ascertaining the function of the morphologically distinct parts of the renal unit.

Deductions as to function. Certain deductions as to function are suggested by reviewing the structure of the kidney in this way. Thus the fact that cilia in the neck segment and in the intermediate segment, when present, disappear coincidently with the acquirement of a relatively high blood pressure in the birds and mammals, suggests a relationship of these cilia to the low blood pressure of the cold-blooded animals. It is probable that in these vertebrates the cilia are needed to aid the relatively low filtration pressure in propelling fluid down the tubule. Again, it seems to be reasonably clear that the ability of a kidney to excrete a urine which is hypertonic to the blood plasma depends on the presence of the thin segment of the medullary loop (Starling and Verney, 1925; Crane, 1927). In elasmobranchs, teleosts, amphibians, and reptiles, the urine is never hypertonic compared to the blood plasma (for literature, see Smith, 1932); in birds, d'Errico (1907) found that the urine could have a slightly higher osmotic pressure than the plasma; and in mammals, as is well known, markedly hypertonic urine can be secreted. Now the thin segment of the loop of Henle appears first in birds, but is completely evolved only in mammals. Indeed, Peter (1909) has correlated the length of this segment in different mammals with the ability of their kidney to excrete a more or less concentrated urine.

It is interesting to note that in the schematic representation of the tubules of vertebrates, the proximal convoluted segment with brush border always follows the glomerulus and neck segment except in the case of the elasmobranchs. Here, the brush border segment is intercalated between two segments, identical in structure, which are ap-

parently not represented in other vertebrate kidneys (Borcea, 1906). Just what this peculiarity means is not known; it may be that it is concerned in some way with the urea excretion, since the elimination of a urine with a much lower content of urea than the plasma is a characteristic of this sub-class. Clarke and Smith (1932) have recently shown that in the dogfish the filtered urea may be almost completely reabsorbed by the tubule.

In view of the fact that more knowledge about the morphology and cytology of the renal tubule in many of the lower vertebrates is needed and that functional studies of many types are almost completely lacking as yet, this discussion must be limited to certain features of the comparative physiology of the kidney.

The aglomerular kidney. The aglomerular kidney was discovered in the Lophobranchs by Huot in 1897 and described by him in 1902. In 1910, Audigé, in a general study of the kidney in teleostean fish, found in the kidney of the goosefish (*Lophius piscatorius*) neither an arterial supply of blood nor glomeruli. The aglomerular nature of the kidney in the Lophobranchs has also been confirmed by Verne (1921–22) and Edwards (1928). The kidney of the goosefish has recently been carefully studied by Marshall and Grafflin (1928) and by Edwards (1928), and the common toadfish (*Opsanus tau*) has also been found to have an aglomerular kidney (Marshall, 1929). The goosefish and the toadfish kidneys will serve as types for a description of this form of teleostean kidney.

Contrary to the statements of Audigé (1910), it has been found that the kidney of the goosefish has a small arterial blood supply and a very few structures resembling renal corpuscles. Definite renal arteries, however, are absent, the main arterial supply being from minute branches of the lateral and ureteral arteries. The main blood supply is almost entirely venous, the afferent venous trunks being the caudal, caudal accessory, sub-clavian, and epibranchial veins. The whole kidney can be injected from any one of the afferent veins. The large posterior cardinal vein is the only efferent vessel. Seventy-eight structures resembling glomeruli were found in one kidney, while an estimate of the number of tubules gave about 150,000 as a minimum, or about 1 pseudoglomerulus to each 2,000 tubules. Grafflin (1929) has shown conclusively that in the adult fish these pseudoglomeruli are non-functional, as careful study by maceration and serial section methods reveals no connection of these structures with a renal tubule. However, in the kidney of a young fish (63 mm. long), he found 94 glomeruli, all

of which were connected to renal tubules. It is, indeed, interesting that these glomeruli should lose their connections with the tubules in the adult fish.

The kidney of the goosefish is composed of great numbers of irregularly convoluted tubules with definite blind endings, surrounded by a mass of pseudo-lymphoid tissue. The tubules may attain a length of 10 mm. or more, and have an average diameter, as measured in section, of about 0.05 mm. Complete vascular injection of a kidney demonstrates freely anastomosing venous channels closely investing the outside of the tubules. The epithelium of the tubule is described by Audigé (1910) as possessing a brush border throughout (with the exception of the initial collecting tubule). Edwards (1928, 1929) gives drawings illustrating two segments, with and without brush border (proximal and distal convoluted tubule), but subsequently, writing of the distal convoluted tubule, he says: "This convolution is present and of considerable length in the kidneys of representative animals in the chief classes of vertebrates except in that of the marine fish" (Edwards, 1933). This agrees with Audigé and makes the goosefish tubule a unisegmental nephron.

The kidney of the toadfish is entirely aglomerular; no structures in the least resembling glomeruli are present. This kidney possesses a very small arterial supply, but the main afferent channels are venous. The tubules resemble those of the goosefish and are surrounded by pseudo-lymphoid tissue. Excluding the initial collecting tubule, it is here certain that only one type of epithelium is present, that of the proximal convoluted segment with brush border. Grafflin (1931) has shown this by a reconstruction of a tubule from carefully prepared serial sections, and Defrise (1932), using refined cytological methods, concluded that only one type of cell is present. Therefore, the whole function of the kidney here is performed by a single type of epithelial cell, corresponding to that of the proximal convoluted segment of the mammalian organ.

The apparent paradox of a kidney having a large number of glomerular structures but showing complete lack of glomerular function by physiological tests has recently been explained by Grafflin (1933). In the kidney of the daddy sculpin (*Myoxocephalus scorpius*) he finds marked evidences of glomerular degeneration and obliteration even in the youngest specimens examined, and in the old specimens it is practically impossible to find a single glomerulus which, from careful anatomical study, can be considered functional.

Comparison of function of glomerular and aglomerular kidney. What is known of the function of the aglomerular kidney can best be understood by describing the similarities and differences which it exhibits in comparison with the glomerular fish kidney (Marshall, 1930). The rate of urine flow of the aglomerular kidney does not appear to be markedly different from that of the glomerular kidney of certain marine teleosts, but is normally much less than that from the well glomerularized kidney of a fresh-water fish. Under as nearly normal conditions as can be experimentally attained, the urine flow of the toadfish is usually below 2.5 cc. per kilogram per 24 hours, while that of the sculpin (a glomerular marine teleost) may be slightly higher (4 cc. or less). How fundamental such a difference is cannot be stated at the present time (Grafflin, 1931). The fresh-water catfish, on the other hand, may have a urine flow of over 300 cc. per kilogram per day.

The urine of both aglomerular and glomerular marine teleosts contains large amounts of magnesium and sulphate, very small amounts of urea, and relatively large amounts of creatine and undetermined nitrogen.[3] Magnesium, sulphate, creatine, and many foreign substances introduced into the fish's body may be present in the urine of both types in much higher concentration than in the blood plasma. The threshold for certain substances exhibited by the glomerular kidney is seen also in the case of the aglomerular kidney. Thus, the urine of the toadfish may contain, under some circumstances, about the same concentration of chloride as the plasma, while under normal conditions it is free of chloride, with large amounts, of course, still present in the plasma. It would appear then that the aglomerular tubule, and, indeed, the single segment with brush border, can excrete a urine of the same composition as that eliminated by the glomerulus and tubule together.

What, then, are the functional differences of the glomerular and aglomerular tubule? The inability of the aglomerular kidney to eliminate glucose and other sugars, its specificity in the excretion of foreign substances, and its ability to secrete against a higher pressure than the blood pressure are the important differences known at present. A rise of blood glucose to over 500 mgm. per 100 cc. is not followed by the appearance of glucose in the urine of the aglomerular fish, while a much

[3] Grollman (1929) finds that the undetermined nitrogen of goosefish urine is mainly trimethylamine oxide and may constitute 28 to 63 per cent of the total nitrogen. Hoppe-Seyler (1930) finds trimethylamine oxide in the urine of selachians, but not in that of fresh-water teleosts.

less degree of hyperglycemia produces glycosuria in glomerular fish (sculpin, eel, flounder, and dogfish). Neither after the administration of phlorizin alone nor in combination with large amounts of glucose can a qualitative test for sugar be obtained in the urine of aglomerular fish. A quantitative investigation of the true glucose in urine has shown that in the urine of the aglomerular fish only traces at most of glucose are ever present.

It appears that none of the sugars are secreted by the aglomerular kidney. Thus, Jolliffe (1930) and Clarke and Smith (1932) have shown that xylose and sucrose, although freely excreted by glomerular fish, are not secreted by aglomerular fish from plasma levels of 300 to 400 mgm. per cent. Lactose is not secreted when injected in large amounts into the toadfish.

The glomerular kidney appears to excrete, to some extent at least, practically all diffusible foreign bodies which may be introduced into the organism; such behavior is, indeed, demanded by the filtration theory of glomerular function. This behavior in regard to foreign substances is not exhibited by the aglomerular kidney; the tubule shows specificity in its behavior to these substances just as the so-called true secretory glands do. A study of the excretion of phenol red and ferrocyanide as two distinct types of foreign substances has been made in representative species of the various classes of vertebrates. While phenol red is readily excreted by the kidney of all vertebrates, ferrocyanide fails to be secreted by aglomerular kidneys. The concentration of phenol red in the urine of the toadfish may be 150 times greater than in the blood plasma, but ferrocyanide when present in the plasma in large amounts can be found in the urine in only faint traces. Phenol red and ferrocyanide are here taken only as types of foreign substances; many others could be used. Creatinine, uric acid, iodides, nitrates, thiosulphates, sulphocyanides, indigo carmine and neutral red are all secreted by the aglomerular kidney, while iron salts and cyanol are not excreted. Marshall and Grafflin (1933) found that injected inorganic phosphate is not secreted by the aglomerular kidney, but that the urine from an aglomerular fish may at times contain large amounts of inorganic phosphate. This paradox is as yet unexplained. Bieter (1931) has also shown that albuminuria cannot be produced in the aglomerular fish by procedures which readily give it in a glomerular fish. Höber (1930) has confirmed this specificity of the aglomerular kidney by showing that after an intravenous injection of a mixture of phenol red and cyanol into the goosefish, the former only is excreted in the urine. He had

previously found that phenol red is secreted by the tubule of the frog's kidney and that cyanol is not.

Recently Bieter (1931) showed that in the aglomerular kidney (toadfish) the secretion pressure taken in the ureter may be greater than the dorsal aortic blood pressure. Since the main blood supply to the toadfish kidney is venous, it appears that the secretion pressure can be many times as high as the blood pressure in the kidney capillaries. The aglomerular kidney thus resembles the true secretory glands. It is safe to predict that in no glomerular kidney can the secretion pressure rise higher than the blood pressure, for even if the tubule can here secrete against a high pressure, back filtration through the glomerulus must occur.

Function of proximal convoluted segment of renal tubule. These studies on the aglomerular kidney of fish and a comparison of its function with that of the glomerular kidney of the same order of animal prove conclusively that the tubule can secrete. The absence of the excretion of glucose and other sugars, the specificity in the excretion of foreign substances, and the ability to secrete against pressure establish the excretion of the aglomerular kidney as being a case of *true secretion.* Since the tubule of the toadfish consists of only one segment (Grafflin, 1931; Defrise, 1932) it is obvious that this proximal convoluted segment can be secretory.

It is unlikely that the secretory activity of this segment is lost entirely when a glomerulus is present. Recent work on the kidney of the sculpin (fig. 1, *3*) has shown that in this glomerular fish the proximal convoluted segment can both secrete and reabsorb (Marshall and Grafflin, 1932). The sculpin kidney can be rendered functionally aglomerular by several injections of phlorizin; after such injections, it no longer excretes substances which are not secreted by the aglomerular kidney—sugars, ferrocyanide, cyanol.

This would appear to be conclusive evidence that the proximal convoluted segment of a glomerular kidney can secrete when glomerular activity is abolished. Does this segment secrete when the glomeruli are active? The urine/plasma ratio for glucose after small doses of phlorizin, used as a measure of the amount of glomerular filtrate, shows that in the marine teleost kidney a large percentage of the magnesium and sulphate of the urine as well as of injected creatinine and phenol red appear to be excreted by tubular secretion.

Since the urine of a normal sculpin is free of chloride and glucose, while the plasma contains these substances, it follows as a corollary of

glomerular filtration that these substances must be reabsorbed by the proximal segment. Reabsorption of water by the proximal segment is proved by the fact that the urine/plasma ratios for glucose and xylose can reach values over 2 (neither of these substances is secreted by the tubule of the sculpin).[4] By direct methods White and Schmitt (1926) had previously localized the reabsorption of chloride and glucose by the proximal segment of Necturus.

Primitive function of the glomerulus and adaptation of glomerular development in animals to habitat. Sixteen species of fish belonging to six families are now known to have aglomerular kidneys (Marshall, 1929). These all belong to the group of marine teleosts. Why do these fish have aglomerular kidneys? This question may possibly be answered by some important work of Homer W. Smith. Smith (1929) finds that in teleosts about 6 to 10 times as much nitrogen is excreted by the gills as by the kidneys. Moreover, he (Smith, 1930, 1932) has found that marine teleosts swallow sea water, absorb from the intestine a large amount of the water and salts, and excrete by means of the gills a much greater percentage of this water and certain salts than is eliminated by the kidneys.[5] Fresh-water teleosts, on the other hand, absorb water from their environment probably by way of the gills and excrete large quantities by way of the kidney. On the basis of this work, Smith has suggested that the aglomerular state of certain marine teleosts is due to their peculiar water cycle resulting in the excretion of only small amounts of water by way of the kidney.[6] If this view is correct, glomerular development should be related to the need for water excretion by the vertebrates generally, and a preliminary survey of the vertebrate kidney has been made from this standpoint (Marshall and Smith, 1930).

The general theory which has been developed may be briefly outlined

[4] Marshall and Grafflin (1932) concluded that the same histological type of cell could both secrete and reabsorb in the sculpin kidney. Since Defrise (1932) has finally concluded that the sculpin tubule contains two cytological cell types (on the basis of Golgi apparatus), Marshall and Grafflin's original conclusion is not justified.

[5] Keys (1931) has reported the direct demonstration of the secretion of chloride and water by the perfused gills of the eel in sea water. Keys and Willmer (1932) find in the gills of the eel and other marine teleosts cells of a secretory type and discuss the possible correlation of these secretory cells with the chloride-secreting activity of the gills.

[6] The recent finding of Armstrong (1933) that the pronephros of the aglomerular toadfish never develops glomeruli is interesting in this connection.

as follows. The excretory organ or kidney of the protovertebrate ancestor of the fish was presumably aglomerular,[7] and in the course of the evolution of the earliest fishes the glomerulus was developed in response to a need for excreting water which was taken up in large amounts from the fresh-water medium in which these animals lived. With a migration to the sea, the need for the glomerulus ceased because water was no longer being taken into the organism in large amount and because the gills could excrete considerable water, due to the difference in the external medium to which they were now subjected. Hence the kidney of the marine teleost has become, or is becoming, aglomerular. All fresh-water fish, however, teleosts, ganoids, and dipnoans, as well as the amphibian still have need of active glomeruli. The reptile has completely eliminated itself from the necessity of an aquatic environment, and has no need for glomeruli unless a new purpose is served by them. The same holds true for the birds and mammals.

If we examine the character of the glomerular development in the different groups of vertebrates, we find that it agrees fairly well with the above hypothesis. In general, we can say that the development of glomerular surface in fresh-water teleosts, ganoids, dipnoans, amphibians and mammals is extremely good, while in marine teleosts, reptiles and birds it is distinctly poor. If we adopt a subsidiary hypothesis that in the mammals (and to some extent in the lower vertebrates) the primitive water-excreting function of the glomerulus has been secondarily diverted to the excretion of waste products, we see that the known facts agree well with the hypothesis.

Relation of comparative data to theories of urine secretion. Let us now see what bearing these fragmentary observations on the comparative physiology of the kidney have upon current theories of urinary secretion. The ability of the glomerular kidney to eliminate to some extent practically all soluble and diffusible substances which may enter the blood stream, and the necessity of the glomerulus for such a process argues for the filtration theory of glomerular function. The abundant other evidence for filtration warrants the acceptance of this theory, with the reservation that possibly the glomerulus may at certain stages of development secrete as well. The high cubical epithelial cells of the visceral layer of Bowman's capsule seen at one stage in the embryonic kidney, the cubical epithelium in certain renal corpuscles of the opossum's kidney described

[7] Nussbaum (1886) and Armstrong (1932) present definite evidence for secretion in the aglomerular pronephric tubule of fish embryos.

by MacNider (1927), similar findings in the kidney of the mouse (Standfuss, 1908) and the frequency with which rather high epithelium on the visceral layer of the capsule occurs in many of the lower vertebrates[8] would appear to justify consideration of the possibility of secretion by the glomerulus.

If we accept glomerular filtration, reabsorption of some substances follows as a necessary corollary, and, of course, there is definite evidence that this process takes place in the tubule and indeed in the proximal segment. That this same proximal convoluted segment with brush border can also transfer substances from the blood and lymph to the lumen of the tubule is proven by a consideration of the morphological and functional studies on the toadfish kidney which have been outlined. The occurrence of this proximal convoluted segment with brush border in the kidney throughout the vertebrates and the improbability of its secretory function being entirely lost certainly suggest that some tubular secretion occurs in all vertebrate kidneys.

Relative importance of filtration, reabsorption and tubular secretion in different vertebrates. Urinary secretion, then, consists of glomerular filtration, tubular reabsorption and tubular secretion. What may be the relative importance of the filtration-reabsorption mechanism and of tubular secretion in any particular kidney and under any given conditions will depend on many factors. It is obvious that in eliminating water, the glomerulus will also eliminate diffusible substances from the blood plasma, and that these will be excreted unless they are reabsorbed by the tubule. It is also clear that where large amounts of fluid are eliminated by the glomerulus, the waste products of the organism may be almost entirely eliminated by this route and hence secretion of the normal waste products by the tubule may be very small. On the other hand, where no fluid is eliminated by the glomerulus, as in the case of the aglomerular kidney, tubular secretion represents the entire renal function. Various intermediate cases will be found depending on the glomerular development of the animal, the glomerular activity under the experimental conditions, and the particular substance investigated.

We can now examine from this standpoint what is known about tubular secretion in different classes of vertebrates. As has been shown above, in the marine teleost, where glomeruli are absent or very poorly developed, secretion by the tubule plays a large rôle in the secretion of urine. In

[8] Osawa (1897) states that in the kidney of Sphenodon the visceral layer of Bowman's capsule is composed of high, cubical cells.

the fresh-water teleost, on the other hand, the enormous quantity of very dilute urine eliminated by the well-glomerularized kidney makes it probable that secretion by the tubule under ordinary circumstances is very slight and requires special experimental conditions for its demonstration.

Nash (1931), from quantitative data on the number and size of the glomeruli in the kidneys of many marine and fresh-water teleosts, concludes that glomerular development is much greater in the latter than in the former. Certain additional data on the glomerular development of marine teleosts are given by Smith (1931).

The amphibian (or at least the aquatic species) resembles the fresh-water fish in eliminating large quantities of very dilute urine. A tremendous amount of investigation has been conducted since Nussbaum's time to test the idea of tubular secretion in the frog. The results have been somewhat conflicting. Richards (1929) and Bieter and Hirschfelder (1929)[9] believe that glomerular filtration and tubular reabsorption are sufficient to explain the excretion of the substances which they studied in this animal, while Marshall and Crane (1924), Tamura and his co-workers (1927), Bensley and Steen (1928), Höber and his pupils [Höber (1929), Scheminzky (1929), Höber (1930)], and Oliver and Shevky (1929) consider that secretion occurs in the proximal convoluted segment of the tubule. Even among those workers who consider that their evidence indicates secretion, there is no general agreement as to what substances are secreted by the frog's kidney. As regards urea, the most abundant waste product in frog's urine, Marshall and Crane (1924) and Höber (1930) interpret their experiments as indicating some tubular secretion of this substance, but Cushny (1926) and Walker and Elsom (1931) conclude that it is not necessary to assume tubular secretion of urea. It has recently been found that the amount of urea excreted by the frog's kidney is greatly in excess of the amount which can be accounted for by the glomerular filtrate as measured by the xylose method. This would make it appear that it is partly excreted by tubular secretion (Marshall, 1932). It may well be that under different experimental conditions the relative amount of tubular secretion compared to glomerular filtration varies widely and that where negative results are obtained

[9] In the light of the studies of Marshall and his collaborators on the aglomerular kidney, Bieter and Hirschfelder speak of the glomerulus as the preferential route for excretion. In a recent note, Richards (1932) states that his more recent experiments indicate "that in the frog secretion is a factor in the elimination by the frog of neutral red but not in that of phenol red."

they are due to inability to detect, by the methods used, the small amount of secretion under the given conditions.

Turning now to a consideration of the birds and reptiles, where glomerular development is extremely poor, we also find evidence for secretion by the renal tubule. Mayrs (1924), from a functional study of the bird's kidney, concludes that a large part of the uric acid is eliminated by tubular secretion, while the very careful and extensive work of Gibbs (1929) upon the same kidney seems to be explicable on no other basis than that there is considerable tubular secretion of uric acid. The very high concentration ratio frequently reached by uric acid, the absence of any relationship between its excretion and blood pressure, renal blood flow or water elimination, the lack of real selectivity in absorption from the tubules, and the increased percentage of uric acid during diuresis are the strong arguments advanced by Gibbs in favor of the tubular secretion of uric acid.

Although Gibbs' evidence appears quite convincing in regard to secretion in the bird's kidney, it may be well to present here certain additional evidence. In the pigeon and the chicken, the renal corpuscles are very poorly supplied with capillary loops; the central part of the renal corpuscle is a dense syncytial-like tissue[10] and the afferent vessel gives rise to only two capillaries on the outside of this central core. The glomerulus, because of this fact and of its small size, can not present a very large filtering surface.

Certain calculations in regard to glomerular filtration in the fowl's kidney may be of interest. The total numbers of glomeruli in the kidneys of two chickens of about 2,500 grams body weight were found to be 840,000 and 848,000, with an average diameter of the renal corpuscles of about 75 microns.[11] We can compare these figures with data for a rabbit of about the same size. A 2,400-gram rabbit was found to have 414,000 glomeruli in both kidneys; the average diameter of the renal corpuscles was 142 microns. Thus, the glomeruli in the fowl are about twice as numerous as in the rabbit and of about one-half the size.

[10] Marshall and Smith (1930) believed they were the first to describe the central core of dense syncytial-like tissue in the avian glomerulus, but I have since found that Li Koue Tchang (1923) noted this some years ago; he called it connective tissue, however.

[11] The following glomerular counts on a few other birds made by Kunkel may be given here. A duck of 3,670 grams had 1,989,000 with an average size of 57 microns in the two kidneys; a goose of 5,400 grams, 1,658,000, with an average size of 75 microns. Six pigeons varying from 232 to 420 grams gave counts of 274,000 to 353,000 in both kidneys.

Hence, allowing for the much less developed vascular tuft in the fowl, it is certain that the total glomerular surface of the chicken is considerably less than that of a rabbit of the same weight. But, on the theory that glomerular filtration furnishes all of the constituents of the fowl's urine, difficulties are encountered. A calculation from the urea ratio of Addis (Taylor, Drury, and Addis, 1923) shows that about 250 cc. of glomerular filtrate per hour is required in a 2,500-gram rabbit to account for the urea eliminated in the urine; about 300–350 cc. of filtrate would be required to account for the excretion of creatinine (MacKay, 1930). In the case of the fowl the figures on uric acid in the plasma and urine require from 1400 to 3300 cc. of glomerular filtrate per hour to explain the uric acid elimination on the filtration-reabsorption hypothesis alone. In other words, about ten times as much filtration is demanded from the filtering surface in the bird, which is much less extensive than that in the rabbit. It is doubtful if differences in blood pressure or colloid osmotic pressure could increase the filtration to this extent, especially as the visceral epithelium of Bowman's capsule in the chicken appears to be much thicker than the same structure in the rabbit.

The situation in the arid reptiles, such as snakes and lizards, is apparently very similar to that in the birds, but practically no functional studies have been made of the reptilian kidney.[12] Here, the small renal corpuscles are poorly vascularized, and hence the total glomerular surface must be small. A comparison may be made of the relative extent of glomerular surface in the horned toad (*Phrynosoma cornutum*) and the common frog (*Rana pipiens*). Hayman (1928) has reported glomerular counts on the kidney of the frog of 1,592 to 5,260 per kidney, the number most frequently being around 2,000. Since the size and weight of the frogs is not given, we can not tell to what extent this factor accounts for the variable counts. We find a 19.5-gram frog to have 1,608 glomeruli; a 26.5-gram frog, 2,220; a 295-gram bullfrog, 6,400; and a 268-gram animal, 8,150. We conclude that large frogs have in general a greater number of glomeruli than small ones, and that for frogs of 20 to 25 grams the number of glomeruli per kidney is about 2,000. Four horned toads, varying in weight from 16.9 to 24 grams, had 1,575 to 2,425 glomeruli per kidney. It is thus obvious that the kidney of the horned toad does not contain more glomeruli than that of a frog of the same body weight. Since the renal corpuscles in a frog of about 20 grams

[12] Bordley and Richards (1932) report that the analysis of glomerular fluid taken from the renal corpuscles of snakes shows the uric acid concentration to be of the same order as in the blood plasma.

weight have an average diameter of about 160 microns and those of the horned toad are only about 65 microns in diameter and have a poor capillary development, it is clear that the glomerular surface in the latter animal is much less than in the former. The total capillary surface of a glomerulus can be taken to vary as its volume if it is entirely filled with capillaries of the same diameter. But the frog's glomerulus is more vascular than that of the horned toad, so this assumption gives too high a value for the capillary surface of the latter. Even on this basis, we find that the total glomerular surface of the horned toad is only about 7 per cent that of a frog of the same size.

An arid-living lizard (*Iguana iguana Shaw*) has recently been investigated. The number of glomeruli in the kidney appears to vary with the size of the animal as is the case with frogs. Thus, an 882-gram specimen had 17,000, a 2,200-gram one, 78,000, and a 2,500-gram one, 121,000 glomeruli in one kidney. The size of the glomeruli was about 70 microns. Considering the size and poor vascularization of the iguana glomeruli, it is evident that the glomerular surface is here much reduced. A comparison of the amount of glomerular filtrate, as measured by the glucose ratio after phlorizin, with the amount of uric acid excreted indicates that in the reptiles, as in the bird, the major portion of the uric acid is excreted by tubular secretion (Marshall, 1932).

In the mammals we find excellent glomerular development. This, together with the complete development of the loop of Henle, suggests that the primitive water-excreting function of the glomerulus has been almost completely modified and the glomerulus now serves as the main route for the excretion of the normal constituents of the urine.[13] This agrees with the idea that in mammals filtration and reabsorption play a major part in the excretion of urine, and that secretion exists only as a relic of a primitive process (Mayrs, 1924; Marshall, 1926). To explain the formation of hippuric acid and ammonia by the mammalian kidney would seem to necessitate secretion by the tubule, and certain other substances also appear to be secreted.

From the work of Jolliffe, Shannon and Smith (1932) on the use of non-metabolized sugars to measure the glomerular filtrate in mammals, it is probable that the filtration-reabsorption mechanism is sufficient to excrete practically all of the normal constituents of the urine. On the

[13] Smith (1932) has pointed out that the filtration-reabsorption mechanism in the mammal does not owe its existence to any superiority of this method over tubular secretion, but is a consequence of the evolutionary history of the vertebrates.

other hand, there is now proof that a very large percentage of certain substances is excreted by tubular secretion in the mammal. It is certain that a large amount of phenol red is excreted by tubular secretion in the dog (Marshall, 1931).[14] Using xylose as a measure of the glomerular filtrate, Shannon, Jolliffe and Smith (1932) have shown that nearly 50 per cent of ingested creatinine may be excreted by tubular secretion in the dog, and Jolliffe and Chasis (1933) find that in the human subject about 42 per cent of ingested creatinine is secreted.

Since the evidence is becoming very strong indeed that glucose after phlorizin or xylose can be used to measure the glomerular filtrate in different classes of vertebrates (Jolliffe, Shannon and Smith, 1932; Clarke and Smith, 1932; Marshall, 1932),[15] we can give a preliminary table of the magnitude of the glomerular filtrate in different animals. These figures are not entirely satisfactory for several reasons. The use of glucose under phlorizin to measure the filtrate is open to the objection that this drug affects the kidney (Marshall and Grafflin, 1932; Clarke and Smith, 1932; Shannon, Jolliffe and Smith, 1932). It is not at all certain that weight or surface area is the proper basis on which to compare different classes of animals. The conditions under which the data for calculation of the filtrate have been obtained are not comparable for all the animals; and, since Shannon, Jolliffe and Smith (1932) have shown

[14] Doctor Shannon informs me that by the use of xylose to measure the glomerular filtrate in the dog, he has found that a large percentage of the total phenol red excreted from low plasma concentrations can not be accounted for by glomerular filtration alone. Chambers and Kempton (1933) have developed an ingenious method of studying renal function by the use of tissue cultures and a micromanipulative technique. They find that the epithelium of proximal convoluted tubules, mesonephric and metanephric, of chicks and young mice can take up phenol red from the surrounding medium and transmit it to and accumulate it in the lumen against a concentration gradient.

[15] White and Monaghan (personal communication) have recently compared in the dog the clearances of xylose, sucrose and glucose, and fail to find as close an agreement as was reported by Jolliffe, Shannon and Smith (1932). Prof. Homer W. Smith, on the other hand, informs me that he and his co-workers have accumulated considerable additional evidence of the validity of the use of xylose or sucrose to measure the glomerular filtrate both in the dog and in the human subject. Pitts has recently found that when the plasma level of inorganic phosphate is raised by injection, the concentration ratios of inorganic phosphate and xylose approach one another closely enough to give an independent check on the measurement of the glomerular filtrate by xylose. The question at issue would appear to be whether the discrepancies between the several sugars are attributable to differences in the way they are handled by the kidney or to technical difficulties in chemical analysis.

that diet can alter the glomerular filtrate in the dog more than 300 per cent, it is obvious that quantitative comparison of the present figures is not justified. Nevertheless, table 2 does emphasize some of the points which have been discussed above. The very low values for the urine flow and glomerular filtrate of the marine teleosts and reptiles stand in contrast to the high flow and high filtration rate in the amphibians and fresh-water teleosts. The relatively low concentration ratios (U/P) for all animals below the bird can be interpreted as indicating a poor water-reabsorption mechanism, while the change to very high ratios, beginning in the bird and reaching its maximum in certain mammals, shows the importance of the loop of Henle for efficient tubular reabsorption of water. Of course, in non-diuretic dogs the glucose or xylose ratio can reach very much higher values than those given in the table.

Blood supply of kidney. A consideration of the blood supply to the kidney of different classes of vertebrates appears to confirm the ideas expressed above. In the aglomerular kidney of the marine teleost the afferent blood supply is almost entirely venous in nature (renal portal systems) and here tubular secretion is the sole process. It is interesting that in all vertebrates (with the possible exception of the Cyclostomes) below the mammals,[16] there exists a double blood supply to the tubule, an efferent glomerular vessel and a renal portal system (Jourdain, 1859; Noll, 1924; Spanner, 1924); this is quite to be expected if tubular secretion plays a greater rôle in the lower vertebrates than in the mammals. It is generally believed that the bloods of the efferent glomerular vessel and of the renal portal system mix freely and supply all parts of the tubule, but the functional investigations of Scheminzky (1929) and Oliver and Shevky (1930) seem to show that this idea of the blood supply of the tubule of the frog's kidney may have to be modified. Their findings indicate that the distal convoluted segment is supplied by the efferent glomerular vessels and the proximal convoluted segment by branches of the renal portal vein. Policard (1910) states, on anatomical grounds, that this is the case. A more complete and careful investigation of this point should be made.

Histological evidences of secretion. Many observers have described what they believe to be histological evidences of secretion in the renal cells of the lower vertebrates. Thus, rather definite granules or globules of material are described as in the process of extrusion from the cells of

[16] Das (1931) states that the bird has no true renal portal system similar to that of fishes, amphibians, and reptiles.

TABLE 2

Urine flow, water reabsorption, and glomerular filtrate

	URINE FLOW	U/P RATIO GLUCOSE OR XYLOSE	GLOMERULAR FILTRATE	REMARKS
	cc. per kilo per hour		*cc. per kilo per hour*	
Elasmobranch: Squalus acanthias (dogfish).	0.18– 1.63	2.15– 6.74	1.21– 4.06	From Clarke and Smith (1932). Xylose
Teleost: Myoxocephalus octodecimspinosus (sculpin, marine)....	0.11– 0.98	0.51– 2.75	0.13– 0.96	From Marshall and Grafflin (1932). Glucose after phlorizin
Ameriurus nebulosus (catfish, fresh water).	6.4 –13.6	1.25– 1.90	10.0 –17.8	From unpublished observations. Xylose
Amphibian: Rana catesbiana.....	1.5 –20.7	1.16– 3.00	2.8 –40.0	From Marshall (1932). Xylose
Reptile: Iguana iguana..	0.20– 1.23	1.5 – 4.9	0.3 – 3.8	From Marshall (1932). Urethane, glucose after phlorizin
Alligator mississippiensis.....	0.43– 1.20	2.6 – 4.2	1.5 – 3.4	From Burgess, Harvey and Marshall (1933). Water diuresis, xylose
Bird: Chicken.....	2.8 – 7.5	4.7 –17.3	23.5 –91.6	From Marshall (1932). Urethane, glucose after phlorizin
Chicken.....	2.0 –17.7	5.2 –18.0	36.6 –93.0	From Burgess, Harvey and Marshall (1933). Water diuresis and pituitary extract. Xylose
Mammal: Dog.....	2.7 –19.3	7.3 –63.2	92 –327	From Jolliffe, Shannon and Smith (1932). Water diuresis, xylose, and raffinose

the tubule and mesonephric duct of *Myxine* (Conel, 1917) and recently Herring (1929) has reported what he regards as histological evidence of secretion in the kidney of the skate. Defrise (1932) and Oliver and Lund (1933) are the most recent writers to present cytological changes in the renal epithelial cells of the lower vertebrates which they believe to be indicative of secretion. The histological evidence for secretion in the cells of the sexual segment of the kidney of male snakes and lizards (Regaud and Policard, 1902–03; Cordier, 1928), and in the renal cells of nest-building fish like the stickleback (Hess, 1918) would seem to be quite definite.

In conclusion it may be stated that this fragmentary account of certain phases of the comparative physiology of the vertebrate kidney appears to indicate the value of pursuing this line of inquiry to obtain an understanding of renal function from a broad biological viewpoint and to properly evaluate much of the work on the lower animals in its relation to renal function in man. From what has been presented, we conclude that glomerular filtration, tubular reabsorption, and tubular secretion all probably occur to some extent in all vertebrate kidneys (except the aglomerular kidney). The relative importance of the filtration-reabsorption mechanism and of tubular secretion of any given substance depends on the substance in question, the particular species of animal under consideration, and the conditions obtaining at the time of observation. In the human kidney, where filtration-reabsorption appears under ordinary conditions to play a predominant rôle for the normal urinary constituents, it is possible that under certain pathological conditions the more primitive secretory process in the tubule may be of major importance.[17]

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[17] This same idea is implied by MacNider (1932) who states that future studies of the details of the circulation in the pathological kidney may alter the conception that an obliteration of glomerular vessels results in atrophy of the tubule. Doctor MacNider informs me by a personal communication that he has very definite reasons to believe that under certain pathological conditions a blood supply is furnished the tubular tissue of the kidney, independent of the glomeruli.

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